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**PROCEEDINGS  
OF THE  
5th ANNUAL CONFERENCE  
ON  
ATMOSPHERIC CONTAMINATION  
IN  
CONFINED SPACES**

16-18 SEPTEMBER 1969

DECEMBER 1969

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AEROSPACE MEDICAL DIVISION  
AIR FORCE SYSTEMS COMMAND  
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## FOREWORD

The 5th Annual Conference on Atmospheric Contamination in Confined Spaces was held in Dayton, Ohio on 16, 17, and 18 September 1969. Sponsor was the Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command. Arrangements were made by the Toxic Hazards Research Unit of SysMed Corporation under the terms of Contract F33615-70-C-1046. The Toxic Hazards Research Unit is located at the Toxic Hazards Division, Wright-Patterson Air Force Base, Ohio and the papers presented at this Conference by personnel of SysMed Corporation represent research conducted under the cited contract. Dr. Anthony A. Thomas, Director, Toxic Hazards Division, served as Conference Chairman. Mr. Edmond Vernot served as Conference Coordinator for SysMed Corporation, and Mrs. Mildred Pinkerton for the Air Force.

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## WELCOMING REMARKS

Clyde H. Kratochvil, Colonel, USAF, MC

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Thank you, Dr. Thomas, and welcome. It was also my privilege to welcome you last year. At that time, though, I had only been assigned to this organization for one month, so I didn't really know too well what I was welcoming you to. This year I do know, and I would like to say that I am very proud to be associated with Dr. Anthony Thomas' group because they are a hard-working, productive group. I want to publicly recognize Senior Master Sergeant John Naylor who has just received the Air Force Commendation Medal for his outstanding work in superintending the construction of the new Toxic Hazards facility which you will visit.

I am pleased and proud that the Air Force has such a group as the Toxic Hazards Division, because this says that we in the Air Force do care about the problem of atmospheric contamination and pollution. We could very easily neglect our role in adding to the burden of pollutants by placing our military role in very narrow perspective. However, we do care and this is attested to by the very considerable resources that we expend in investigating the various problems caused by our exotic missile fuels, etc. To phrase it quite candidly, we 'put our money where our mouth is', and in these days of austerity this is saying quite a bit.

Lastly, I would leave you with just a philosophical reminder: At this Conference you will be discussing confined spaces and the contamination of those spaces. Those of us who feel we are responsible citizens, responsible scientists, are convinced, as I am sure you are, that when we are talking about confined spaces we are also talking about the Planet Earth and the biosphere which we occupy. Unless we - you, I, and the rest of the scientific community - attack this problem and attack it vigorously, we are going to destroy the ecological niche which we and most other living things occupy. This cannot be stressed too strongly.

With this I will cease preaching and introduce to you my personal friend of long standing and currently my chief, the Commander of the Aerospace Medical Division, Major General Charles Roadman.

### INTRODUCTORY REMARKS

Charles H. Roadman, Major General, USAF, MC

Commander  
Aerospace Medical Division

Dr. Thomas, Colonel Kratochvil, ladies and gentlemen: I have been asked to make the keynote comments to this symposium.

I noticed a recent article in the Federal Times dealing with problems of overpopulation. In this article it predicted that at the present rate of growth the world population will increase from 3.6 billion in 1970 to 7.5 billion by the year 2000. This is a staggering figure and is a major problem as we all know.

This is more than double our present world population in the next 30 years. At the present rate of contamination and pollution of our atmosphere, those of us who are still around 30 years from now may be spending all of our time looking for some clean air to breathe, or probably we'll have a very difficult time in getting a controlled sample of pure air to compare.

After our astronauts landed on the moon someone made the statement that they didn't think the moon would be able to support life. Well, it's getting more and more difficult here on earth, as we know. Perhaps this is a very simple way to outline our concerns at this symposium.

Seriously, we all know this is a major problem. You are all aware of it. I'm sure that I don't have to convince this group of the seriousness of the situation, and certainly the federal government, state governments, and many of our municipalities are spending a considerable amount of time attacking this problem. Because of this, meetings of this type take on an added significance and certainly the work that will be discussed during this meeting will contribute to the solution or will, at least, point up some of the increasing dangers of our present rate of air pollution.

I certainly hope that this symposium can contribute to actions which are urgently needed in this great country of ours. I hope that while you're here you will take advantage of the opportunity to visit our recently expanded Toxic Hazards facility at the Aerospace Medical Research Laboratory. I had the occasion to go through this facility a few weeks ago for the first time after its completion, and I was very very pleasantly surprised and pleased with this new laboratory. We are very proud of this expanded facility and of the new capability that it gives us.



The toxic hazards research accomplished at the Aerospace Medical Research Laboratory over the years, like other areas of research within the Aerospace Medical Division, played a significant role in this nation's ability to land man on the moon. In the area of toxicology there were over 200 non-metallic space cabin materials screened for composition and toxicity of off-gassing products. Tolerance limits were established for ethylene glycol vapor in a space cabin atmosphere as well as tolerance limits to carbon monoxide. Studies were conducted on the toxicity of pulmonary irritants and systemic toxicants in ambient air and in space cabin atmospheres.

The laboratory conducted extensive studies on the mechanism of oxygen toxicity at reduced pressures and compared ultrastructural changes in the lung, liver, and kidney under 5 psi oxygen and the same pressure of mixed gas atmosphere.

With the completion of additional facilities in the Toxic Hazards Division, they will be capable of performing toxicology research beyond the closed environment area and into the study of human performance during chemical stress in high altitude or at sea level. We feel that this is a very important adjunct to this research. The human performance aspect is one which receives more and more emphasis in modern toxicology as a most sensible end point in seeking tolerance levels in the absence of overt chemical symptoms or pathological change.

The design of these new altitude facilities has inherent advantages for the execution of human exposure studies which cannot be found in any other existing inhalation chamber complex. The concept of performing physiological and performance measurements during exposure thus can be carried out in an optimum fashion which makes this facility highly attractive to any agency engaged in research of public health or environmental pollution problems.

We will now be capable of a much higher degree of sophistication in carrying out toxicological research into related ultrastructural, biochemical and pathological studies than has ever been previously possible. Common sense tells us that the facilities of the Toxic Hazards Division of the Aerospace Medical Research Laboratory, like many other facilities within the Aerospace Medical Division, will be a national resource. It is a resource with a tremendous capability to examine long term, continuous exposures to toxic materials in any environment such as aircraft cabin, missile launch complex, ground shelter, and perhaps even the problems encountered in substandard dwellings.

In looking over the program for this fifth annual conference I see a list of names that I recognize, and it appears that you have a full three days ahead of you. A conference of this type always also provides an opportunity to renew old acquaintances and to discuss mutual problems with your counterparts in other organizations.

Now I would like to just make a few remarks in closing. I had the opportunity recently to attend a Laboratory Directors' Fund meeting in Washington chaired by Mr. Hansen, who is Assistant Secretary of the Air Force for Research and Development. There were two significant things that came out during this meeting which I think are

important to this group and give us a few indicators of the future. The first concerns a definition of the type of work. I think that we are all aware of Congressional interest and scrutiny of research and development. It is becoming increasingly important to define and describe proposed research in understandable terms. The use of simple descriptors and avoidance of technical jargon is imperative today. The fund managers at high levels must be assured of the mission relevance of proposed research.

Another point arose from this conference, and it was quite pointed, and many of you are aware of this. The Defense Department is coming under ever-increasing scrutiny and ever-increasing criticism in certain areas. I'm not here to defend or debate or support the particular issue, sometimes referred to as the military-industrial complex, but the Defense Department is being forced to scrutinize closely its research. Specifically, Air Force research and development must be clearly defined as having a complete and defensible Air Force need. In addition, the need to reexamine our types of research was emphasized. When there is commonality in research, for example, with NIH or some other Federally supported research agency, the other organization and/or agency should support it rather than the Air Force.

These last remarks should be of particular concern to you in this room. Is air pollution a singular responsibility of the Air Force? This question will be asked and must be answered.

In addition to this we are faced with ever-increasing manpower reductions. We find that we no longer can take manpower cuts across the board in our research laboratories, and we're having to look at the priority of entire programs rather than try to hold the line with a minimum number of people. We feel that it's better to eliminate the program rather than to continue in a mediocre fashion.

There is a need to look at joint utilization of government laboratories by the universities of this country. This effort is already under study by direction of the President. This is a matter of national concern.

In summary, we must now learn to manage laboratories with greatly reduced resources and markedly changed mission requirements.

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SESSION I

TOXICOLOGICAL EVALUATION OF ATMOSPHERES  
AND CONTAMINANTS

Chairman

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## METHODS OF HUMAN PERFORMANCE MEASUREMENT IN TOXIC ENVIRONMENTS

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The question of performance measurement in toxic environments is certainly not new; yet never has there been such widespread interest in this area. This surge of interest can be traced back to two sources. First, the logically more important task of establishing a methodology for determining clinically "safe" dosage levels has been developed to such a degree that we now have a certain amount of confidence in the methods of pathological investigation. Along with this, we are seeing increased accuracy and completeness of epidemiological records.

The second development lending itself to increased concern with performance deals with the nature of the tasks we are requiring the human operator to perform in modern systems. For better or worse, we are requiring humans to operate at near peak potential in a variety of performance modalities for long periods of time. In today's high performance aircraft and space systems, as well as in our high speed and ultra-responsive automobiles, even a momentary lapse in efficiency can lead to ultimate disaster. Taken together, these factors have tended to change the basic question which is being asked in toxicological research. We now ask not only "does this agent damage the individual?" but also "does this agent affect the individual at all?"

In view of the urgency of this question, it is somewhat disappointing to review the literature specifically relating low-level toxicity to what may broadly be called "performance". Even the relatively few studies that can be found reveal little homogeneity among the kinds of performance tasks used. More importantly, there is little attempt to relate the studies to any theoretical framework, let alone any real-world systems requirement. Tasks appear and disappear from study to study with confusing regularity. The experimental design and data treatment frequently show almost naive simplicity. Control groups are inappropriate or, worse, not used at all. No attempt is made to use double-blind techniques where they are appropriate and clearly feasible. Statistical and design controls which would bolster validity (such as independent replications, trend analyses, and powerful factorial models) are not even considered. The design itself is often weak and does not give adequate attention to extraneous variables such as fatigue, order effects, subject preselection, or interaction of the toxic agent with other stressors inherent in the testing situation. In addition, one sometimes encounters a study from which broad, sweeping generalizations have been made and

which, on inspection, shows statistical treatment which is so naive and inappropriate as to make the entire result invalid.

There are probably many reasons for this lack of an overall approach to performance measurement. In the specific case of toxic environments, the psychologists and human engineers who would be responsible for such methodological considerations have not had enough control over the experiment because of the medical aspects which had to be given precedence. More generally, the blame must be attributed to the fact that the psychologists and human engineers have failed to give an adequate test to several generalized methodologies which are available. This paper will summarize one of these methodologies, and discuss one of the more general questions surrounding the choice of a particular way to measure performance. The approach to be discussed is not presented as being the only, or even the best, answer. It does, however, provide a clear illustration of how the complex question of performance measurement can be broken down into manageable elements, while still retaining application to the real world.

One of the most powerful techniques available for evaluating the effectiveness of performance tests is that of factor analysis. Although the details of this approach are beyond the scope of this discussion, it is possible to understand the basic logic of factor analysis in the following way. Suppose several tests are given to a number of individuals and all the possible correlations between these tests are determined. As illustrated schematically in figure 1(A), if two of these tests show no correlation at all, i. e., there is no relationship between an individual's scores on the two tests, we can be reasonably certain that each test measures a different skill. By a more complex but similar reasoning (figure 1B), if several tests correlate highly it can be established that they measure the same or related skills. Thus, if many tests are given to many subjects, it is possible to arrive at the position illustrated in figure 1C where a small number of "test clusters" have been developed. Each cluster consists of several tests which measure the same skill to various degrees, and each cluster measures a different skill than every other cluster. Within each cluster of tests, it is frequently easy to identify the underlying skill which is being tapped. For instance, we may discover that every test in a cluster has an element of reaction time in it. In this case, we would have reduced a number of apparently disjointed performance tests to one specifically defined skill - simple reaction time. Extremely sophisticated mathematical treatments are necessary to determine these factors, and the system is not without its weak points. But the fact remains that it is a systematic and exhaustive attempt to define empirically the nebulous term "performance", and that it forces the experimenter to define clearly what he is testing.

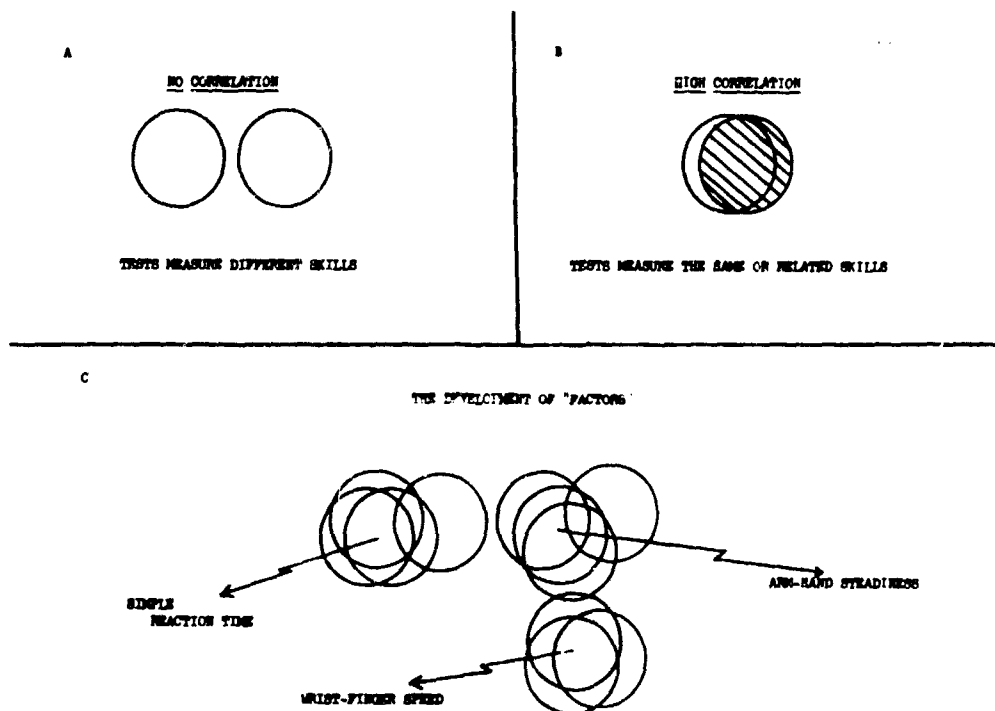


Figure 1. SCHEMATIC REPRESENTATION OF FACTOR ANALYSIS

Using just such techniques, Fleishman and his associates have administered over 200 tests to thousands of subjects and have broken down psychomotor performance into a number of discrete skills or "factors" (Fleishman, 1954; Fleishman, 1956; Fleishman, 1957; Fleishman, 1960; Fleishman & Hempel, 1954; Fleishman & Hempel, 1954; Fleishman & Hempel, 1956). These factors include control precision (involving highly controlled large muscle adjustments), multiple-limb coordination, response orientation (involving rapid directional discrimination and orientation of movement patterns), simple reaction time, speed of arm movement, rate control (making continuous anticipatory motor adjustments relative to changes in speed and direction of a moving target), manual dexterity, finger dexterity, arm-hand steadiness, wrist-finger speed, aiming, strength, flexibility of muscle groups, and balance.

The most important point is that for each of these identified factors we have at our disposal a series of empirically defined tests. We know what each test measures in terms of the kinds of test performance which are correlated with it, and we know how well it measures those performances. The experimenter is thus given a freedom to choose the test that best suits his experimental environment, and his results will still be interpretable to other investigators who have chosen different tests.

The relationship between these factor-analyzed performance tests and more complex real world performances such as flying have been the subject of extensive study. In many cases, the relationship is so obvious that we can identify it immediately. For instance, Fleishman's factor of multiple-limb coordination is obviously one of the skills involved in accelerating a standard shift car. In other cases the relationship is not so obvious. Then we must again conduct studies to determine correlations between the real world skills and the factors. These may be predictive studies, such as when we predict on the basis of high scores in a set of factors that certain people will do well on a real task, or they may be retrospective studies where we test a number of real skills and see which factors correlate with each skill. In either case, we have taken the total complex task, whether it is assembly of small components on a production line or piloting a space craft, and we have broken it down into a small number of independently tested skills which are essential to good performance on that task.

Given this repertoire of available tests, how does the investigator make the final determination of which skills he will test and what particular measures he will use to test them? Obviously, there will be environmental and monetary limitations on what and how much he can do. Subjects may be partially restrained during exposure. Certain types of apparatus or data reduction capability may not be available. In these cases, it is sometimes necessary to choose tests which may not be the best or factorially "purest" measures of a particular skill. However, even in these situations, we still know how good the test is, and by adjusting the experimental design - say by increasing the number of subjects tested or using an additional measure - we can compensate for the initial lack of precision.

The choice of which skill to test is generally dependent on the type of output information desired from the experiment. In general, there are two types of questions we are asked in toxicological research. In the first type, we wish to determine the effects of a particular agent on a given real-world skill. This would be the case if we wished to know the effect of marihuana on automobile driving. In this case, the experimenter would first analyze the task involved and determine which skills are essential to that task. Again, this is sometimes a rather straightforward procedure. However, in some cases, especially in the absence of previous factor-analytic research on that task, this would require making some hypotheses. But even in this difficult situation, the rationale behind the hypotheses would have to be clearly defined, and the tests used could readily be interpreted by the field as a whole.

In the more general, more frequent case, we are asked simply if there is any effect at all due to a given agent. Fortunately, in most cases, something is already known about the physiological effects of the agent, and often animal studies have already defined approximately the types of skill that are degraded by large doses. Using this type of information, it is possible for the experimenter to describe in general terms the limits of performance skills which could be affected by low levels of the agent. In carbon monoxide exposure, for instance, there is ample evidence that higher nervous processes deteriorate first. In this case, we could "bound" the entire range of expected effects by testing primarily cognitive functions and those psychomotor skills which require a great deal of cortical integration. Such cognitive skills have also been subjected to factor analysis, and therefore form a complete spectrum of skills ranging

from the most cortically dependent down to those which involve only gross muscular movement or strength. In our laboratory, using carbon monoxide and working under a time constraint, we chose one highly cognitive task - time estimation, one complex psychomotor task - tracking, and one rather complex, subcortically mediated task - dynamic equilibrium. In addition, we further tested for lower order effects by monitoring galvanic skin response, heart rate, and respiration. In this way, we were in a better position to make conclusions not only about the specific tests we used, but also to propose hypotheses about the entire range of performances covered by our tests.

Given the above rationale for a more molecular approach to performance testing, it might now be appropriate to consider a possible objection. Why go to all the trouble of factor analysis, or any of the other more molecular approaches, when the easiest thing to do would be to create a simulation of the real world task? If we are interested in piloting an airplane, why not use a simulator, or even better, take our subjects in a plane? Although this would not be easy in view of the dangers involved, there is a certain magic about such "face validity" and vast amounts of money are spent in making sure that a test "looks like" what it is supposed to measure.

Unfortunately, long experience has taught that the validity of such studies often ends at its "face". Basically, the problem appears to involve the fact that when a complex task is taken in toto into the laboratory setting or is made part of an experiment, the elements of the task no longer interact the way they do in the real world. It has been possible to achieve fairly high predictive validity with such work sample tests only when the real-world task was not very complex or when we were willing to invest a great deal of time on a very specific task with little generalizability. As soon as the task becomes complex, as most real tasks are, we find in most cases that the task variables are interacting so strongly with uncontrolled factors in the testing situation that our measurements are meaningless. The lesson from all this is that when we try to duplicate a real-world performance, we run a great risk that the task we end up with will be a different breed of animal from the one we started out to measure.

With a more molecular, independent skill approach, we have constrained the subjects' behavior in such a way as to obtain a "purer" measure of each skill without losing our ability to interpret interactions among skills. The experimenter has thus gained a great deal of control over the variability shown by subjects. In research involving very low levels of toxic substances, where the effects which can be expected are not extremely large even when they occur, this can be a crucial statistical determinant in evaluating the significance of results. In this regard, certain extremely sophisticated developments in the area of psychomotor tracking performance have recently been made by Jex and his associates at Systems Technology Incorporated (Jex, 1967; Jex, McDonnell, & Photak, 1966). It now appears that with proper mathematical analysis and modeling, it is possible to constrain not only the operator skill which is being tested, but even his method of attacking a given problem. Full realization of this potential will enable us to specify not only that a given agent has affected a particular skill, but to isolate where, in the sequence of events from stimulus to response, the decrement appeared.



In summary, it has been proposed here that, when human performance is tested in toxic environments, detailed consideration should be given to the rationale behind selection of the tests to be used. Specifically, it is felt that use of the molecular approach, based on empirical criteria of skill definition, provides the greatest freedom to the experimenter and retains the greatest interpretability from one study to another. Several generalized methodologies of this type are available, only one of which has been presented here. Use of such approaches should do much to alleviate the existing confusion and contradiction regarding the question of low-level toxicological effects on human performance.

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## DISCUSSION

MAJOR THEODORE (Aerospace Medical Research Laboratory): I'll try to field Dr. O'Donnell's questions, and I may have to use some consultants here - Dr. Mikulka, Lt. Heinig, if necessary.

DR. PIERSON (Lockheed-California Company): The author advocates molecular behavior, molecular testing of molar behavior, and to date this has never worked simply because the sum is equal to more than the sum of the parts. The total behavior is not a collection of small parts, and I couldn't refuse commenting about factor analysis. Please, people, when you use factor analysis, there are three things to keep in mind. Number one is that when you're all done you have a series of four to five factors which have to be identified and can be identified only by intuition. The purpose of statistics is to eliminate intuition. Secondly, that, in factor analysis, linear correlation is assumed for all variables and this is rarely true for human performance. And thirdly, that you only get out of a factor analysis what you put into it. If you put garbage in, you get garbage out. Fleischman, for instance, in all of his studies, has never identified isometric strength, isotonic strength, or endurance as part of performance, simply because he never had those kind of tests put in to it to begin with.

MAJOR THEODORE: I may need help from one of my consultants, but let me answer this in part. I think there's too much research done where people just look at the general issue and don't try to find out what is going on. For example, you say a man breathes, by your analogy; therefore, we shouldn't find out about what energy pathways there are that make him subsist. I think the same thing is lacking in psychological studies. There is lack of definition in the discipline in many respects, and I think many amateurs get involved in the area, don't try to find out the basic methods, pathways, in order that you can put it all into one operational system. You can't understand how the total human behaves if you don't understand its individual components. Therefore, being a non-psychologist, this type of approach is appealing to me. I think that one can't make generalized statements when you don't understand the components that are involved in that statement. Just like describing any system, superficial observations never work. They give you an immediate answer, but it doesn't really solve the problem until you start to get some understanding. I realize that there are many shortcomings since systems haven't been developed yet. However, I think that this should be the aim of the future. This is exactly what I think Dr. O'Donnell is trying to say here, that we should try to define exactly what we are doing so that data from different labs are comparable, so that we know exactly what we did. Certainly, often, the experiments are fraught with great errors and great inconsistencies because people haven't broken down the components in trying to analyze them, and this is the only plea I make here. Maybe Dr. Mikulka would like to answer certain aspects of your question.

DR. MIKULKA (Old Dominion University): I think what we have done is to take the best technique in a very bad area and try to make it more rigorous, so that as Dr. Theodore mentioned, all diverse studies that are being done in the area of contaminants, at least some results come out and you can face the problem, "Why did he get this when I got that?" It's not a good measure, maybe, but it's the best we have right now.

MAJOR THEODORE: I think the key here is trying to define exactly what you have done. I think this is just good research, and I think that when one establishes exactly what is going on, what mechanisms are involved, then you can be a little bit intelligent about your analogies and your approach to the problem. This is just my feeling.

DR. HODGE (University of Rochester): Not being a psychologist, I certainly don't want to get into this discussion about the whole being greater than the sum of the parts, but I wonder how many of you happened to read the article of Lord Brains in Science just a few months ago. The title of the thing was Science and Anti-science. But, in part, he discussed something that is down underneath here. He pointed out, quoting Aldous Huxley, that literature describes our emotions, our intuitions, how we react, why we react, in private, in terms of our private experience, which we cannot and never will reveal; whereas science describes our public reactions and what can be checked and double-checked and confirmed. Is this part of our problem?

MAJOR THEODORE: I would say yes.

## THE EFFECT OF CARBON MONOXIDE ON HUMAN PERFORMANCE

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### INTRODUCTION

Carbon monoxide (CO) has become an important byproduct of increasing industrialization. The potential risk of this toxic substance applies not only to the general population, but also to specific subpopulations (i. e., respiratory and cardiovascular patients, military, etc.). This risk is considered extremely important in the aerospace environment where human performance is carried to its extreme limits in high performance aircraft and space systems. Any effect of low levels of CO on performance must be considered serious insofar as it affects the system operator. Mechanisms of CO action must be correlated with performance decrements in order to arrive at realistic guidelines on atmospheric control for man.

The central nervous system is extremely sensitive to oxygen deprivation and is considered the primary locus of CO induced effects, since the major mechanism of CO action is mediated through tissue hypoxia (Dinman, 1968). It is generally believed that subjective symptoms rarely occur below carboxyhemoglobin (COHb) levels of 20 percent, while most acute signs of cardiovascular, respiratory, and central nervous system embarrassment occur at COHb levels greater than 30 percent (Haldane, 1927). However, a number of investigators have indicated that the central nervous system is impaired at COHb levels as low as two to five percent. MacFarland, Roughton, Halperin, and Niven (1944) demonstrated impairment of visual discrimination with COHb levels of four percent. Lilienthal and Fugitt (1946) reported impaired flicker fusion at an altitude of 6000 feet with COHb levels of five and ten percent. Decrements in limb coordination have been shown with the same COHb levels at ground level atmospheric pressure (Trouton & Eysenck, 1961). Consistent impairment in cognitive and psychomotor performance has been noted by Schulte (1963) at five percent COHb, and some tendencies for disruption as low as two percent COHb. Finally, Beard and Wertheim (1967) have shown decreased auditory discriminability of tone lengths with COHb levels of approximately four to five percent.

However, not all investigators have shown such effects at low levels of COHb. Clayton, Cook, and Frederick (1960) found no association between COHb level and automobile accidents. Dorcus and Weigand (1929) found no decrements in cognitive and psychomotor tasks with COHb levels as high as 25 to 35 percent. Vollmer, King, Birren, and Fisher (1946) found no changes in flicker fusion, visual perimeter, or ataxia as a function of COHb levels up to 22 percent. However, their study was run at simulated 10,000 and 15,000 foot altitudes, and the hypoxic contribution of those altitudes may have masked possible CO effects.

Cigarette smokers are estimated to have a constant COHb level between 3.8 and 6.8 percent. Further, 12 to 14 percent of employed individuals have occupations in which there is a likelihood of exposure to high levels of CO (Goldsmith and Landaw, 1968). Given these facts, plus the studies which suggest decremental effects under COHb levels that the average smoker carries within his system, one could conclude that a large proportion of our population is operating at a depressed level of performance. It therefore becomes important to determine what effects are present, their mechanism of action on the organism, and the importance of these effects on specific performances (i.e., driving an automobile, piloting an aircraft, etc.).

Obviously these questions cannot be answered until a better understanding and correlation of the physiologic, biochemical and psychologic processes of the brain are known. At the present level of sophistication, Dinman (1968) contends that cerebral function should not be impaired at COHb levels of five percent or lower. If this assumption is true, then those data which show decrements with COHb levels of three to five percent must depend on some complex interaction or summation of elements which have yet to be defined.

The present study was undertaken to determine the effects of CO on relatively simple applied performance tasks. Analyses were planned which would reveal whether: (1) absolute performance levels changed as a function of CO exposure, and if (2) CO exposure changed the pattern of performance.

## METHOD

### Subjects:

The subjects (Ss) used in this study were 10 male university students between the ages of 19 and 22 years. All Ss were examined and medically certified to be in good health. All Ss were supposed to be non-smokers. However, after completion of this study, examination of the blood COHb levels indicated that one S was probably a smoker. He confirmed this when questioned and his data were excluded from all analyses.

### Environment:

All CO and control exposures were carried out in the Thomas Domes of the Toxic Hazards Division, Wright-Patterson Air Force Base, Ohio. The domes are a completely enclosed environmental system into which a given contaminant can be introduced and maintained at a given level. Air flow is controlled by a series of blowers and vacuum pumps, which produce a flow of 40 ft<sup>3</sup>/min, yielding a complete atmospheric change every 20 minutes. Each dome is roughly circular with a 12 foot diameter. Ss inside the dome can see into the surrounding room, but for the present study about eight feet of the dome windows were covered in order to eliminate distracting background movement from the S's field of view. However, it was considered important that during "rest periods" the Ss could see outside in order to preclude sensory restriction effect which could mimic or confound CO effects. Temperature was controlled between 68 and 74 degrees F, and dome pressure was held at 680 mm Hg during the experimental runs. Entrance to the domes was accomplished through an airlock, which allowed the interior environment to be kept stable.

### Experimental Measures:

Time Estimation: During each testing interval a series of estimates of a 10-second "empty" interval were made by each S. The S was asked to estimate 10 seconds, beginning on a signal from the experimenter (E). At the end of this estimated interval the S tapped an electronic switch and immediately began estimating another 10-second interval, etc. The E stopped this sequence when the S's last estimate exceeded the three minute test period. All the S's estimates were automatically recorded.

Critical Instability Tracking Task (CITT): The CITT requires the S to stabilize a statically unstable controlled element by closing a compensatory loop around the system (Jex, 1967). Essentially, the S is required to keep a needle on a display dial from going off scale by manipulating a control stick. Referring to figure 1 it can be seen that any output from the integrator will be fed back through the summing amplifier in such a way as to cause the output of the integrator to increase. If the S, by moving his control stick, generates input to the summing amplifier which exactly cancels the input fed back from the integrator, the output (needle on dial face) will remain stationary. Any slight error in timing or the amplitude of the response will cause the output to change. The S's optimal strategy is to keep his control stick displacement exactly proportional to the system output (needle deflection). For the purpose of this experiment the gain control was set to increase linearly over time. In essence, this multiplies the output from the summing amplifier returning to the integrator, causing increased input to the needle, which requires the S to make greater and quicker compensations. As the gain increases a point will be reached where the S cannot possibly respond quickly and accurately enough to "maintain control". This point of control loss is converted into a difficulty level score, and serves as the basis for evaluation of the S's tracking ability. The CITT has been analyzed on theoretical grounds to be sensitive to a number of stressors including hypoxia, drugs, g-levels, low temperature, and secondary workloads (Rosenberg and Jex, 1966; Jex, 1967); and on the basis of the

describing functions involved appears to be most closely related to performances requiring a great deal of perceptual-motor coordination and high requirements for speed and accuracy.

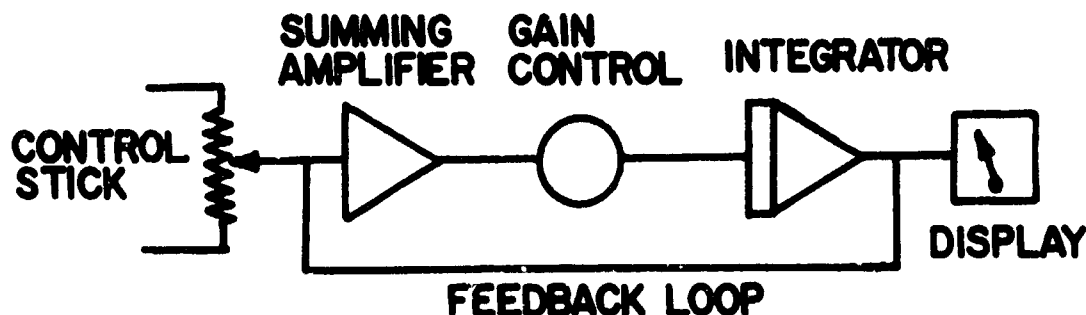


Figure 1. DIAGRAMMATIC SCHEME FOR CRITICAL INSTABILITY TRACKING TASK (CITT).

Pensacola Ataxia Battery: Since one of the effects of relatively severe CO exposure is the loss of balance and other vestibular symptoms, the Pensacola Ataxia Battery was used. This battery consists of a number of balancing tasks performed either on narrow rails or on the floor (Greybeil and Fregley, 1965). For the present experiment the following tasks were used:

1. The Sharpened Rhomberg task (SR) in which the S attempts to stand for 60 seconds in a heel-to-toe position with his arms folded and eyes closed.
2. Walk Eyes Open test (WE/O) in which the S attempts to walk a 3/4 inch wide rail with his arms folded and eyes open.
3. Stand Eyes Open test (SE/O) in which the S attempts to stand on the above rail for 30 seconds with his arms folded and eyes open, and with his feet in a heel-to-toe position.
4. Stand Eyes Closed test (SE/C) in which the S attempts to stand for 60 seconds on a 2-1/4 inch wide rail in a heel-to-toe position, arms folded, and eyes closed.
5. Stand on One Leg Eyes Closed test (SOLEC) in which the S tries to stand on one leg, eyes closed, and arms folded for 30 seconds. This is done alternately on both legs resulting in SOLEC-Right and SOLEC-Left.
6. Walk On Line Eyes Closed test (WOLEC) in which the S attempts to walk a 12 foot line with his arms folded and eyes closed in a heel-to-toe manner.

Design and Testing Procedure:

Each S spent three sessions in the Thomas Domes at 0, 50, and 125 ppm CO. The order of exposures was counterbalanced to avoid possible sequence effects. With the removal of the smoker and due to an error on the part of the technician controlling the exposure contaminant, which resulted in one S being run a fourth time, the resulting order was: three Ss with a A-B-C presentation order (0-50-125 ppm), three Ss with C-A-B, two Ss with B-C-A, and one with B-A-C.

A double-blind procedure was used throughout this experiment. At no time did the Es know what was the atmospheric composition in the dome. Also, all immediately involved support technicians were included in the double-blind coverage.

Ss were scheduled at either 8 AM or 1 PM depending on their availability. Exposure time began at the moment the S entered the dome. Headphones were given the S through which he was in continuous touch with the Es. After a final check of all recording and communication equipment the E left the dome through the airlock. The S was then instructed to relax for a few minutes. The first series of measurements was taken after the S had been in the dome exactly 15 minutes.

A performance session consisted of five trials on the CITT, three minutes of time estimation, and then five more tracking trials. This sequence usually ran from 13 to 16 minutes. After completion of the session the S was instructed to relax for about 15 minutes until the next testing session.

Each dome exposure lasted three hours. The Ss were tested 15 minutes out of each half hour, resulting in the following test intervals after dome entrance: 15-30 min, 45-60 min, 75-90 min, 105-120 min, 135-150 min, and 165-180 min. After 90 minutes of exposure the E entered the dome allowing the S to walk around and stretch. This was done to reduce the possibility of fatigue and boredom from being in a constant position for three hours.

At the completion of each run the S was immediately removed and 10 ml of blood was taken for hematocrit, hemoglobin, and COHb determinations. Then, the S was taken to an adjoining room and given the Pensacola Ataxia battery. Following this the S was asked to breathe 100 percent oxygen for ten minutes.

Following completion of the main study five Ss were exposed to 200 ppm CO and three Ss to 250 ppm. The procedures were basically the same with the following exceptions: (1) the double-blind was no longer in effect since these runs were made for exploratory purposes, (2) counterbalancing was no longer possible, and (3) the Ss were specifically told that they would be receiving higher levels of CO. Because of the lack of counterbalancing and the reduced sample size no analyses were performed on these data.



## RESULTS

Carbon Monoxide Exposure Levels:

Carboxyhemoglobin (COHb) determinations were made on the venous blood for each S after every experimental session. These determinations were done by a modified gas chromatographic method of Dominguez, Christensen, Goldbaum, and Stembridge (1959). The results of the COHb analyses are presented in table I. It can be seen that the COHb levels reflect a direct relationship with the level of ambient CO. These data are in remarkable agreement with the CO uptake curves based on time, exposure concentration, and rates of ventilation constructed by Forbes, Sargent, and Roughton (1945). The exception to this was S #10 who had a COHb level of 3.5 percent in the 0 ppm condition. This S later admitted he was a smoker. His level of 3.5 percent COHb falls into the range of 2.3-3.8 percent COHb reported for light smokers (Ringold, Goldsmith, Helwig, Finn, and Schuette, 1962). Hematocrit levels for all Ss ranged from 42 to 52%, and hemoglobin levels ranged from 12.8 to 17.6 gms percent with no significant changes due to CO exposure.

TABLE I  
CARBOXYHEMOGLOBIN LEVELS FOR ALL SUBJECTS AT EACH EXPOSURE  
(PERCENT)

SUBJECT	0 PPM	50 PPM	125 PPM	200 PPM	250 PPM
1	0.7	2.8	6.5		
2	1.2	2.4	6.6		
3	0.8	2.7	6.5	9.8	
4	1.0	3.1	7.4		13.1
5	1.0	3.0	6.8		
6	1.2	3.6	6.8	10.1	11.9
7	1.1	3.3	6.4	10.0	
8	0.9	3.0	6.6	10.9	
9	0.6	2.9	6.2	10.9	12.1
10	3.5	4.5	7.4		
MEAN*	0.96	2.98	6.64	10.35	12.37

\* does not include values for S #10

Subjective Reports:

All Ss were interviewed informally after each run to determine the incidence of subjectively perceived symptoms. In the 39 experimental sessions carried out, only one S reported any symptoms. This occurred in S #4 during exposure to 250 ppm, who reported a "slight" headache which he said did not interfere with his performance. Further, none of the Ss displayed an ability to indicate whether or not CO had been present during a given experimental run.

Critical Instability Tracking:

The mean difficulty level was obtained for each set of ten trials for every test interval. Therefore, each S had six scores for each exposure level and these were the basis for the analyses.

Since the uptake of CO within the body is a cumulative process over time, it is important to compare not only the differences among the conditions at each test interval, but also to evaluate the trends occurring over the three hours of exposure. Grouped data representing the mean scores for nine Ss at each test interval are presented in figure 2. Initially, it can be seen that no trend toward poorer performance appeared in any of the conditions. In fact, there was a general tendency for all conditions to improve over time. Therefore, each condition was tested independently to determine if performance changes had occurred. These analyses yielded F ratios of 1.84 (for 0 ppm), 1.81 (for 50 ppm), and 2.23 (for 125 ppm), with  $P = >.10$ ,  $>.10$ , and  $<.10$ , respectively. Thus, when considered separately no condition showed a clearly reliable change in tracking performance over time, and the one condition that showed any change at all indicated that tracking performance became slightly better during exposure to 125 ppm. Reference to figure 3 shows that the Ss tracking under 200 ppm and 250 ppm did not differ from their control runs under 0 ppm. In absolute terms, these findings answer one of the primary concerns of this experiment: No decrement in tracking performance was found as a result of exposure to CO levels up to 125 ppm, and quite possibly as high as 250 ppm.

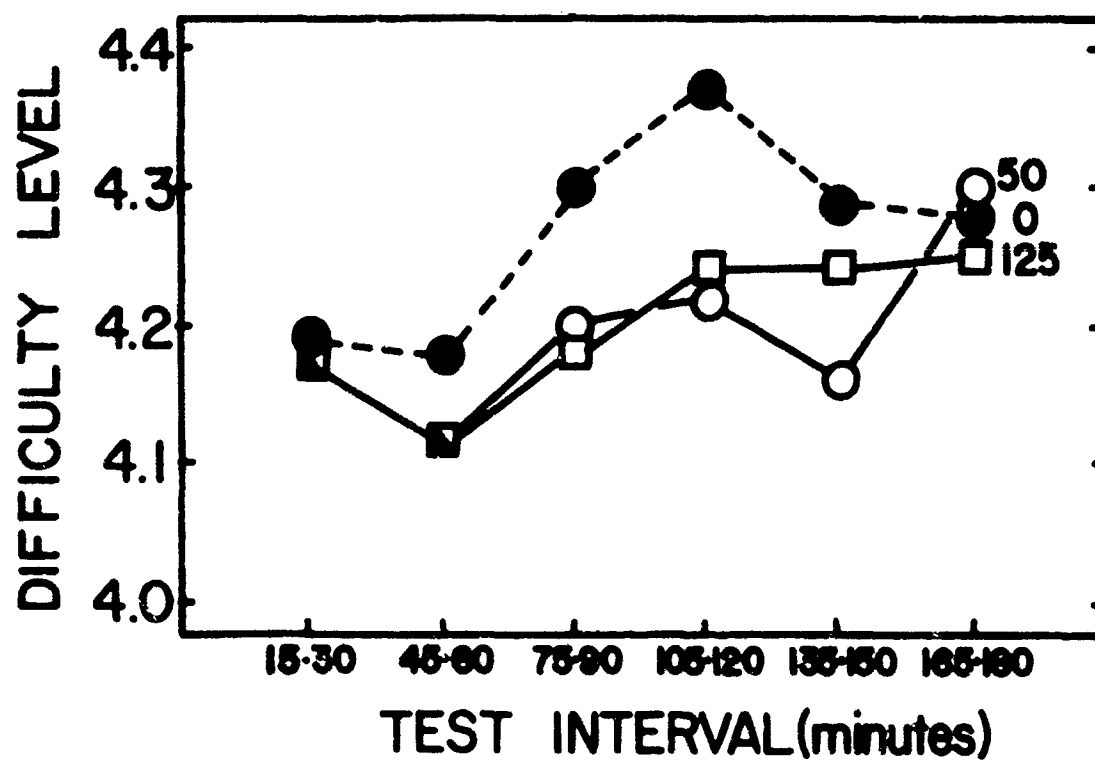


Figure 2. MEAN SCORES FOR CRITICAL INSTABILITY TRACKING TEST (9 Ss)

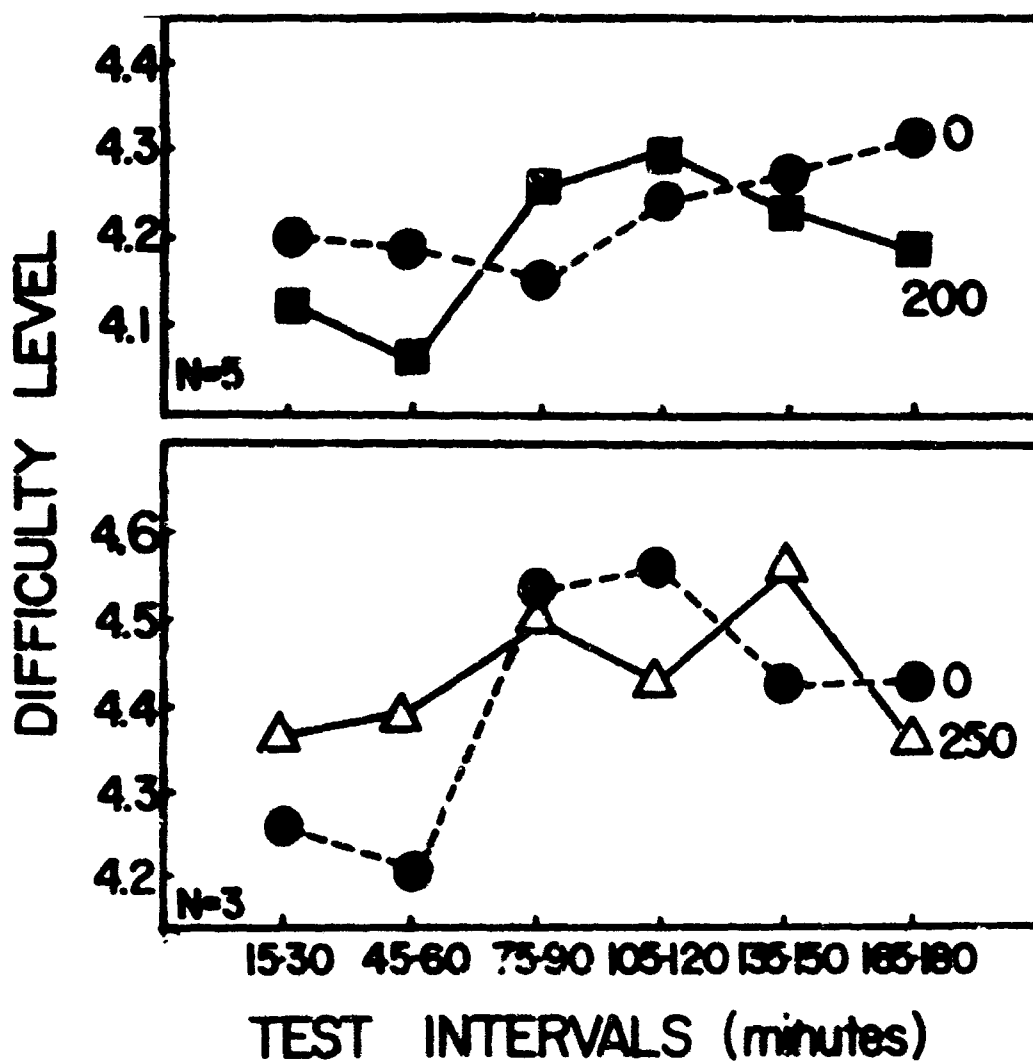


Figure 3. CRITICAL INSTABILITY TRACKING TEST (8  $S_a$ )

In order to determine if there was a relative difference between scores with and without CO present, these data were subjected to repeated-measures-analyses of variance at each test period. The results of these analyses are presented in table II. It can be seen from these analyses that there was a significant difference in performance levels of these three conditions at the 105-120 minute interval, which decreased by the 135-150 interval and completely disappeared by the last interval. Newman-Keuls analyses (Winer, 1962) were performed on the 105-120 and 135-150 intervals. These indicated that performance under 0 ppm was better than either of the CO conditions at the former interval ( $P < .05$ ), while performance under 0 ppm was only marginally better than the 50 ppm condition, though not different from 125 ppm at the latter interval. At both intervals the two CO levels did not perform significantly different from each other. These results suggest that CO exposure resulted in a relative performance change compared to the control condition. However, this effect was transitory reaching a maximum at 105-120 minutes and diminishing as duration of exposure increased. Further, examination of the performance trends shows that these effects were not due to any decrement in the absolute level of performance under CO, but the result of a transitory increase in performance under 0 ppm (see figure 2). Trend analyses were done on each condition over the six test periods (Winer, 1962). The results are presented in table III. These analyses indicate that a large proportion of the 0 ppm trend was composed of linear and quadratic components, at 50 ppm the linear and quartic components composed the largest proportion of the curve, and at 125 ppm the linear component accounted for most of the trend with a smaller cubic component. Examination of figure 2 shows that there are distinct differences in the shapes of the curves and that these are most notably evident in a stronger tendency for linearity over time in the 125 ppm condition and in a much larger quadratic or singly-humped performance over time in the control condition than in any other. Also, average performance at 50 ppm shows a greater tendency to be erratic as evidenced by the large quartic component.

Inspection of the individual performance curves revealed no consistent differences as a function of CO exposure. In four of the nine  $\bar{S}$ s tracking was generally better under 0 ppm than in the other conditions. In only one  $\bar{S}$  was tracking under 125 ppm consistently the poorest, while four  $\bar{S}$ s performed worst under 50 ppm.

As CO uptake increased over time, performance would be expected to show a decrement if there were any simple relationship between the two. However, in no  $\bar{S}$  was an overall time-related decrement seen in any of the CO runs up to and including 250 ppm. In fact, several  $\bar{S}$ s show a remarkably consistent improvement in tracking as a function of time.

TABLE II

## ANALYSES OF VARIANCE ON TRACKING SCORES FOR EACH TEST INTERVAL

INTERVAL	SOURCE	df	MEAN SQUARE	F RATIO
15-30	Between $S_s$	8	.220	.03
	Within $S_s$	18	.020	
	CO	2	.001	
	Residual	16	.023	
45-60	Between $S_s$	8	.218	.41
	Within $S_s$	18	.025	
	CO	2	.011	
	Residual	16	.027	
75-90	Between $S_s$	8	.389	2.35
	Within $S_s$	18	.017	
	CO	2	.035	
	Residual	16	.015	
105-120	Between $S_s$	8	.358	4.53**
	Within $S_s$	18	.018	
	CO	2	.058	
	Residual	16	.013	
135-150	Between $S_s$	8	.410	2.86*
	Within $S_s$	18	.016	
	CO	2	.039	
	Residual	16	.014	
165-180	Between $S_s$	8	.412	.66
	Within $S_s$	18	.009	
	CO	2	.006	
	Residual	16	.009	

\* significant at .10 level

\*\* significant at .05 level

TABLE III  
PERCENT OF GIVEN POLYNOMIAL COMPONENT PRESENT IN TREND ANALYSIS

COMPONENT	0 PPM	50 PPM	125 PPM
Linear	37.9	45.0	62.4
Quadratic	31.3	1.0	0.9
Cubic	9.4	0.9	24.7
Quartic	5.8	41.6	11.6

#### Time Estimation:

Under the procedure for time estimation in this study each S made a series of estimates during each test interval. The mean of these individual estimates was taken for each interval and these means constituted the raw data for subsequent analyses.

The mean performance curves for time estimation under the three experimental conditions are presented in figure 4. It is evident that although there is some separation between the different conditions, no overall trend to over- or under-estimation occurred as a function of CO uptake. There is a slight tendency for all conditions to increase accuracy over time. A very similar pattern can be seen for the Ss exposed to 200 ppm and 250 ppm (figure 5). Under all conditions time estimation remains remarkably consistent.

In order to test for relative differences among the 0 ppm, 50 ppm, and the 125 ppm conditions, separate repeated-measures-analyses of variance were performed. The results are presented in table IV. The only significant difference occurred at the 135-150 minute interval, where time estimates under 50 ppm were longer than under the control condition, as determined by the Newman-Keuls test. Inspection of figure 4 reveals that this difference resulted from a decrease in the average estimates in the 0 ppm condition, and not from any change (away from the real 10-second interval) by the Ss while under CO. The difference dealt with here is less than one second, and it is difficult to construe this as a significant distortion due to CO exposure, especially when this effect was not seen in the 125 ppm condition. At no point were the estimates under 125 ppm different from those under the control condition.

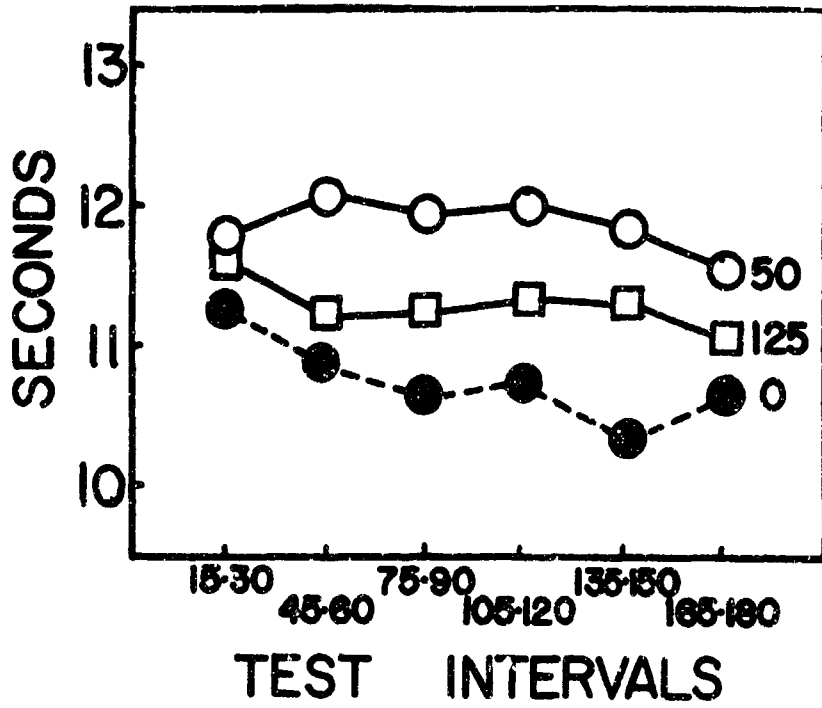


Figure 4. TIME ESTIMATION PERFORMANCE CURVES (0, 50, 125 ppm CO)



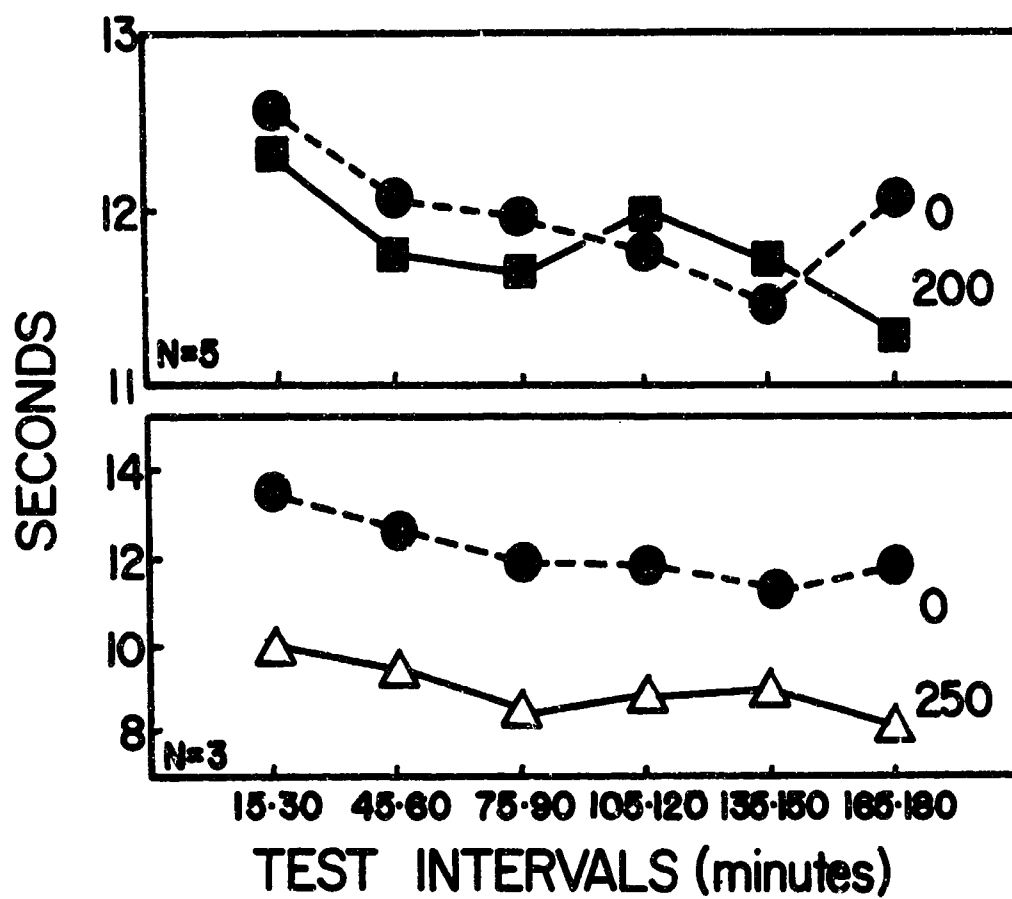


Figure 5. TIME ESTIMATION PERFORMANCE CURVES (200 and 250 ppm CO)

TABLE IV  
ANALYSES OF VARIANCE ON TIME ESTIMATION AT EACH TEST INTERVAL

INTERVAL	SOURCE	df	MEAN SQUARE	F RATIO
15-30	Between $\bar{S}s$	8	14.795	0.121
	Within $\bar{S}s$	18	3.718	
	CO	2	0.500	
	Residual	16	4.120	
45-60	Between $\bar{S}s$	8	13.109	0.824
	Within $\bar{S}s$	18	3.678	
	CO	2	3.090	
	Residual	16	3.751	
75-90	Between $\bar{S}s$	8	15.806	1.417
	Within $\bar{S}s$	18	2.678	
	CO	2	3.605	
	Residual	16	2.562	
105-120	Between $\bar{S}s$	8	18.201	1.663
	Within $\bar{S}s$	18	2.230	
	CO	2	3.455	
	Residual	16		
135-150	Between $\bar{S}s$	8	21.069	3.987*
	Within $\bar{S}s$	18	1.668	
	CO	2	4.980	
	Residual	16	1.249	
165-180	Between $\bar{S}s$	8	27.064	1.468
	Within $\bar{S}s$	18	1.233	
	CO	2	4.989	
	Residual	16	1.172	

\* significant at .05 level.

Inspection of the individual  $\bar{S}$  estimates similarly revealed no consistent effect attributable to CO. In five  $\bar{S}$ s time estimates were longer under CO conditions than under 0 ppm. However, for the other  $\bar{S}$ s one or both of the CO conditions gave more accurate estimates than the control condition. In two cases 125 ppm produced the longest estimates, while in five  $\bar{S}$ s the 50 ppm condition produced the longest estimates.

Further support for the lack of any CO effect on time estimation can be assumed from the performance curves of those  $\bar{S}$ s that received additional runs at 200 ppm and 250 ppm (figure 5). From these curves it is obvious that (1) there is a general tendency for estimates to improve over time, irrespective of contaminant level, (2) performance for the 200 ppm condition closely parallels that for its control run, and (3) under 250 ppm the  $\bar{S}$ s are more "accurate" in their estimates than under the 0 ppm condition, but more importantly there is no indication of a differential effect of CO over time.

In summary, it may be concluded that under the conditions of this study, no effect of CO levels up to 125 ppm and possibly as high as 250 ppm could be discerned on time estimation. In all conditions the estimated 10-second interval was longer than the real 10-second interval.

#### Pensacola Ataxia Battery:

The scoring on the Ataxia battery was accomplished using procedures recommended by Greybeil and Fregley (1965).

The means for each of the tests are shown in table V along with the F ratios from the repeated-measures-analyses of variance. All the resultant F ratios were non-significant. Examination of the means for the respective ataxia test shows that there are no major differences within the means for a given test. In absolute terms, four tests (SR, SOLEC-L, SOLEC-R, & WOLEC) yielded better performance under the CO conditions than under 0 ppm. In only one case (SE/O) were the scores under the CO conditions worse than the control condition.

In view of these results, it is clear that CO exposure had no effect on the kinds of abilities measured by these tests.

TABLE V  
MEANS AND F RATIOS FOR ALL PENSACOLA ATAXIA TESTS

TEST	0 PPM	50 PPM	125 PPM	F RATIO
SR	204.00	221.00	217.00	0.758
WE/O	14.00	14.13	13.75	0.117
SE/O	32.25	29.75	30.88	9.090
SE/C	81.13	80.75	81.13	0.001
SOLEC-L	133.13	138.50	135.13	0.103
SOLEC-R	140.50	150.00	141.50	1.681
WOLEC	17.13	13.38	10.50	0.963

### DISCUSSION

This study attempted to answer the question: does CO affect performance? In order to explore this question, a range of performance measures were taken: 1) time estimation of an "empty" 10-second interval, 2) tracking, and 3) ataxia. On an ordered scale these measures run from the heavily cognitive task of estimating time in which the S supplies his own counting stimuli, to the tracking task which requires the co-ordination of visual input with rapid motor responses, to a psychomotor task involving vestibular and gross motor controls for dynamic equilibrium. The results of this experiment indicated that three hours of exposure to CO levels up through 125 ppm produced no decremental effect in functioning and no consistently reliable pattern changes from the control conditions. Also, there is an indication that carrying the CO levels up to 200-250 ppm will produce no observable effect on the present battery of performance tasks.

The results of this study conflict with several other studies which have found performance decrements under low levels of CO. Beard and Wertheim (1967) using a temporal auditory discriminability task (perceptual) found major disruptions under 50 ppm CO. Unfortunately, they were unable to obtain accurate COHb determination, but given their exposure times it is possible to assume that their Ss did not have COHb levels above 5 percent. It should be noted that their Ss were confined in a soundproof audiometer booth with a total volume of 110 cubic feet. The Ss had no outside visibility and the tasks did not involve a great deal of kinesthetic, proprioceptive, or visual input to the Ss. In view of the fact that even moderate degrees of sensory or motor restriction can cause significant perceptual and motor distortions (Schultz, 1965), it is possible that any CO effects reported could be accounted for, or at least confounded, by sensory restriction effects. In contrast, Ss in the present study were confined in a large dome with an approximate free volume of 600 cubic feet. At all times the Ss could see out-

side the dome. At the midpoint of each run the Ss were allowed to get up and move around inside the dome. Additionally, the tasks involved a good deal of visual input and a significant amount of motor output from the Ss. These procedures were specifically intended to minimize any possible effects of sensory restriction or boredom, in order to yield a less contaminated estimate of CO effects. In view of this it may not be surprising that the present results do not show the perceptual errors seen by Beard and Wertheim (1967).

Schulte (1963) using a battery of psychomotor-perceptual tasks found performance disruptions with reported COHb levels comparable to those obtained after three hours of exposure to 125 ppm CO in the present study. However, there is reason to believe that Schulte underestimated the levels of COHb in his Ss, since he reports obtaining mean COHb determinations of 0.00 percent from Ss who were predominantly smokers, working in a large metropolitan area as firemen. Recent data indicate that 1.2 percent COHb is found in the average metropolitan non-smoker (which agrees well with the 0.96 determination in our non-smokers under 0 ppm CO), while the range for smokers in a metropolitan area runs from 2.3 to 6.8 (light smokers, noninhalers to heavy smokers, inhalers). Further, his findings of perceptual-cognitive decrements conflict with a study which failed to find any decrements of similar tasks with up to 25-35 percent COHb (Dorcus and Weigand, 1929). Also, examination of some of the reported decrements in cortically mediated tasks, such as arithmetic errors shows that the absolute number of errors at 1 percent COHb was higher than at all other levels up to 20 percent, except at two levels.

Trouton and Eysenck (1961) reported impairment in control precision and multiple limb coordination when COHb levels exceeded 5 percent, whereas Schulte (1963) found no change in muscular coordination as measured by reaction time, static steadiness, muscle persistence, and cranial reflexes. Consistent with this the present study failed to find any evidence for disruption in tracking or ataxia with average COHb levels of 6.6 percent. Several other studies failed to find disruption in performances which required a great deal of motor coordination until high levels of COHb were reached (Clayton, Cook, and Frederick, 1960; Forbes, Dill, DeSilva, and Van Deventer, 1937; Rockwell and Ray, 1967; Vollmer, King, Birren, and Fisher, 1946).

In summary, it can be said that the case for performance changes under low levels of carbon monoxide is not very compelling, and must await further experimental support. However, if there is no effect at these low levels of CO on performance, then we have a finding of significant practical importance.

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## DISCUSSION

DR. MACFARLAND (YORK UNIVERSITY OF CANADA): I know nothing about the background of this work other than what you told us today, but as a toxicologist who's done a great many experiments over 25 years or so, as I look at your graphs there's a suggestion that comes to me--it may be valueless here, but I'll throw it out for what it's worth. In looking at the various graphs you have presented of your results, it seems to me, superficially, two kinds of things. In some cases your curves are sort of intertwined and it is perfectly evident there's no significant difference in the CO groups versus the zero group. In some of the other graphs, however, you notice that the curves parallel each other but they are separated by a distance. This usually suggests to me, in the kind of work that I do, that there is probably some kind of systematic error that I've got in my studies, but--again, I throw this out as probably not true--it might be a difference in your analysis for carbon monoxide, or something wrong with the analytical instrument that throws it off by a certain increment. As I say, I don't suppose this is the case here, but the suggestion that comes out of this is that in some of the cases where the curves are separated, more or less parallel, --a good guess would be that there may be systematic error operating that you haven't identified.

DR. MIKULKA: On your point there, there are two things. Statistically, if you see differences in graphs that aren't held up, you really have no basis for saying anything. That's one thing. Secondly, one of the tasks, where you see the big differences in time estimation, was done because somebody else had used it and shown effects. Actually, the study was done with the assumption that there are effects there and we want to see just how big they are and what direction they are. Well, it turns out that the task of time estimation, although you think that everybody has an estimation of what ten seconds is, is a learning phenomenon that, as a function of exposure these guys got better, and even though the design was counterbalanced, a couple of subjects really showed improvement and happened to occur in the "wrong" groups, the CO exposed groups. It only takes one or two subjects who are consistently better to pull that whole thing off. You don't see it as markedly or at all in tracking performance. Everybody is trained very well in specific skills like piloting an aircraft, very complex motor tasks, where they trained maybe for 30 hours apiece. I don't know if that answers your question.

MAJOR THEODORE: I would like to answer the second part of his question about the question of analytic error. Roughton, who probably knows more about carbon monoxide uptakes in hemoglobin than anyone else in the world, constructed curves a number of years ago with Forbes as a senior author, and on his curves he took into account the rate of carbon monoxide passage through the lung, rate of uptake with hemoglobin.



By comparing over the time interval, since carbon monoxide uptake is related to time, the affinity of hemoglobin and rate of ventilation, he constructed these curves, and our data hit his curves on the point almost consistently to the point of disbelief. And the spread of our data was so small that as far as analytical error being part of this, I really doubt this, and theoretically it followed the prediction curves put forth by Roughton.

DR. BACK (Aerospace Medical Research Laboratory): Did you put anything on the board showing carboxyhemoglobin levels?

MAJOR THEODORE: No. I can give them to you; I know them.

DR. BACK: Maybe the audience would be interested in what they were for comparative purposes with Beard's work and others.

MAJOR THEODORE: Well, quickly, Goldsmith and Landau found 1.2% in the average metropolitan nonsmoker; we found 0.96%. At 50 ppm CO we had 2.96%. At 125 ppm CO we had 6.6%; at 200 ppm CO we had 10.35%; and at 250 ppm CO we had 12.37%. The last two points representing only 5 and 3 subjects might be less accurate, but the spread, for instance with normal nonsmokers at zero conditions, (mean was 0.96%) spread was less than 0.1, the total range. Nice and tight, except for the smoker and he was way off. He had a 3.7%, I believe, at zero exposure.

DR. HODGE: May I ask how long it takes for the carboxyhemoglobin curve to get up to near its 3-hour value?

MAJOR THEODORE: As far as our study was concerned, the 3-hour value that we got was predicted by the 3-hour value plotted by the Forbes-Roughton curves. To reach equilibrium, I can't say for sure. I would guess, and this is only a guess on my part, that it would take at least over 24 hours to reach equilibrium. But, prior to that time, it's rate of uptake of carbon monoxide associated with the affinity with hemoglobin, and you have to take other factors into account--ventilation, cardiac output, probably pH effects in the blood. You probably don't reach steady state carboxyhemoglobin level at exposure to a certain carbon monoxide level in the environment until probably after one day of exposure, but this is only a guess on my part. But before that time, it is really an hourly increment.

DR. MIKULKA: I think it's extremely difficult to estimate with the low levels that we are using, 50 ppm, how long it would take or how high it would go.

MAJOR THEODORE: For any of you who would like to look at the curves, there is one in the Handbook of Physiology and Respiration, Volume II.

DR. BENJAMIN (NASA, Washington): Do you consider the effect of carbon monoxide as a specific toxic effect or is it an indirect anoxic effect?

DR. MIKULKA: The mechanism is supposed to be--tissue hypoxia. That's all I can offer as a psychologist, and from what I read from medical specialists in the area, it should be a hypoxic phenomenon, basically. It may be a lot more than that. We don't show any effect, at this level.

CAPTAIN HICKS (U.S. Army Research Institute on Environmental Medicine): I was curious about your choice of a trial by trial analysis of the data, particularly in the time estimation task. I wondered if you would have a different result if you would analyze all the data together.

DR. MIKULKA: We did. We did everything possible. You see, initially, biased like all experimenters who repeat previous work, we said, "We know there's an effect there", and we analyzed everything. We did what Pierson says is dangerous. We analyzed everything. We found nothing.

DR. PFITZER (University of Cincinnati): As you undoubtedly know, there's a great deal of activity regarding setting environmental quality standards for carbon monoxide, and much of this is based on some of the work with which your data do not agree, and undoubtedly, this will lead to the need for further experiments to see if they duplicate your work. I wonder if you have some thoughts on different kinds of performance tasks which you think might be of more value if further work on this question were to be done.

DR. MIKULKA: I think the problem there becomes what areas are these operational limits being set? The population as a whole? For specific aerospace systems? Cardiovascular patients? If you go to space systems, you should use tasks like the pilots of a probable spacecraft on a trip to Mars (which is being projected) would be required to be performed, and I can't be specific unless you throw me a task or type of environment. For the psychologist, for the general population, I'd be concerned with tasks involving intellectual functioning, whether these mental functionings are sharp. All data on intellectual functioning, per se, involve crossing T's in a letter, and reduction to absurd limits, and it's a bad area. That's why factor analysis was suggested. A lot of things should be done that haven't been done.

DR. BACK: I think one of the strong points of this particular experiment is the fact that we used a tracking task at which the individual exposed could not win. The faster he tasked himself the more the guts of the system made him fail. The better he got, the worse he could get, so that he was working against a real handicap. He had to fail, and this, I think, gives you some indication of the fine muscular coordination needed, and the intensity at which the man had to work. This is a fine task for a toxicological parameter in evaluating pilot performance.

DR. MIKULKA: This task was tested and confirmed to be a fairly accurate estimation of tracking performance in auto driving, and in piloting high performance aircraft. It's a very involved tracking test. I think it's a good one, not just simply following a little stylus around, but requires rapid reaction to a dial, very quick responses, and visual input with motor output. It's very difficult, I think.

MR. BIBIE (General Electric Company): This series of tests was what I would call a short term series of tests. It was only three hours. How would your test results change if you ran them for a longer time like 24 hours?

DR. MIKULKA: I think the answer to the question is we don't know. For one, we don't know the uptake curves. They haven't been established yet. At three hours, there are no effects. We go as high as 12.37% CO Hb and there are no effects observed. As a psychologist I would say a man could go, 24 hours under 50-125 ppm and show no observable effects, in the types of tasks we have here. Whether you can go for 500 days, back and forth on a spacecraft mission for instance, is another story, and I think it should be tested, at least for some longer terms of exposure.

MAJOR THEODORE: If we did a similar study, as Dr. Mikulka just described, and ran it over 24 hours (and we might) we would be dealing with a different situation because the carboxyhemoglobin levels would be much higher than they were at any given point, but if you go to the higher levels, like he pointed out, 13% carboxyhemoglobin, and were able to go back to the lower levels at equilibrium, I would say maybe you could go at 50 ppm indefinitely if the sole factor involved in decrement were the carboxyhemoglobin level. You have to try to relate it to what you have available, but if you run experiments longer, over 24 hours, it's just really a function of how high the carboxyhemoglobin levels are going to go and they have already reached their limits depending upon the environment, and some of our high points really give credence to our low points as far as exposure is concerned for long periods of time. I would be willing to say that at 50 ppm, from what little I know, some would probably go on indefinitely.

## EFFECTS OF CARBON MONOXIDE ON THE PERFORMANCE OF MONKEYS

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### INTRODUCTION

The United States Air Force has a very high interest in the short- and long-term effects of carbon monoxide (CO) since studies of gas-off products of space cabin materials and processes (including man's contribution) reveal that the compound is likely to be found in space cabins if not properly scrubbed. In addition, there is a strong possibility for the presence of CO and other agents such as gasoline fumes during loading and unloading of large cargo aircraft as well as under other closed operational conditions where munitions may be used. The experiment reported here is part of a series of efforts to determine the levels of CO necessary to change the performance of trained animals and to correlate such changes with other clinical parameters. It was found in previous experiments that animals exposed to 55 mg/M<sup>3</sup> for 105 days followed by 7 day consecutive exposures to 110, 220, and 440 mg/M<sup>3</sup> CO produced performance changes in 2 of the 12 monkeys tested (at the 110 mg/M<sup>3</sup> and above levels) and that the 440 mg/M<sup>3</sup> CO level also produced changes in appetite and in outward appearance in 6 of the 12 animals (Back and Dominguez, 1968). Since this was a sequential experiment, a decision was made to test the effects of CO on animals that had never before been exposed to CO or other contaminants at a level which might provoke performance changes.

### METHODS

The subjects used in this experiment were 12 adult *Macaca mulatta* monkeys which were trained at Arizona State University and shipped to Wright-Patterson AFB. All animals were trained on the performance tasks to a stabilized level during a period of several months.

The subjects were placed in 12 individual working cages especially designed to fit in front of each of 12 windows within one Thomas Dome in the Toxic Hazards Research Unit. The psychopharmacology programming equipment is solid state and has been described previously in detail (Wolfle, 1966 and Back, 1967). In short, however, the following procedures are used.

The performance panel in each cage includes two stimulus lights over each of two response levers (left and right). These levers are used for a continuous avoidance task. Mounted between and slightly above these levers are two response push buttons. The upper push button contains a white lamp which flashes for a discrete visual response, while a lower button is the response object for a discrete auditory response to a 2800 cps tone mounted at the upper left of the panel.

The animals are programmed to work eight hours each day. They work for 15 minutes each hour starting on the hour, five days per week, for as long as the experiment is in progress. They do not work on weekends. The animals are fed at the end of the last session on each work day. When blood is obtained or when any other procedure involving the handling of the animals takes place, it is scheduled before the first work session in the morning (0800).

Each 15-minute work schedule includes three tasks. The monkeys must press each right and left continuous avoidance lever at least once each 15 seconds or they receive a light shock. In addition, they are presented 12 randomized visual and 12 randomized auditory cues during each 15 minute work period. The response to these cues must be made within two seconds or the animals receive shock. The data are collected and recorded for each session of each work day, and means and standard deviations are determined for each animal. In addition, grand means are made on all animals on a weekly basis to determine performance changes.

Routine clinical laboratory tests (table I) were performed at appropriate intervals before, during, and following exposure to the experimental conditions. Carboxyhemoglobin levels were performed by a gas chromatographic method (Dominguez et al, 1957).

TABLE I  
ROUTINE CLINICAL LABORATORY TESTS

<u>Hematology</u>	<u>Chemistry</u>
WBC	Sodium
RBC	Potassium
Hemoglobin	Calcium
Hematocrit	Total Protein
Differential Count	A/G Ratio
(when needed)	SGOT
	SGPT
	Alkaline Phosphatase

The experimental exposure conditions are outlined in table II. The animals were placed in the Thomas Dome under ambient conditions for a period of 40 days during which time baseline performance and clinical data were obtained. The ambient conditions consisted of an air atmosphere, at 720 mm Hg pressure, air flow rate of 40 cfm, 70-78 F, and relative humidity of 50%. Another baseline or control session was obtained when the animals were taken to altitude (260 mm Hg) in air atmosphere of 68% O<sub>2</sub>/32% N<sub>2</sub> for a period of 28 days. All other conditions previously noted remained the same. Next, CO exposure was begun and continued for 103 days at a level of 220 mg/M<sup>3</sup>. The CO atmospheric content was continuously monitored by infrared spectrometry. Following exposure, the animals were again returned to the ambient condition of air at 720 mm Hg where they were observed for 30 days.

TABLE II  
EXPERIMENTAL EXPOSURE CONDITIONS OF 12 TRAINED MONKEYS

	<u>Atmosphere</u>	<u>Altitude</u>	<u>Duration</u>
1.	Air	720 mm Hg	40 days (3 Feb - 14 Mar)
2.	68% O <sub>2</sub> /32% N <sub>2</sub>	260 mm Hg	28 days (15 Mar - 11 Apr)
3.	68% O <sub>2</sub> /32% N <sub>2</sub> plus 220 mg/M <sup>3</sup> CO	260 mm Hg	103 days (12 Apr - 23 Jul)
4.	Air	720 mm Hg	30 days (24 Jul - 22 Aug)

#### RESULTS

Hematological changes produced by the 103 day exposure to 220 mg/M<sup>3</sup> CO are shown in table III and graphically depicted in figures 1-3. In essence, total red blood cell count, hemoglobin content and hematocrit increased during the exposure period. Peak levels of each parameter were attained approximately one month into the exposure with rbc's increasing by approximately a million, hgb by 2 gm %, and hct by 10 vol %. Concurrently, carboxyhemoglobin levels reached a mean of 22% saturation. These levels plateaued within the first bleeding period (10 days) and remained essentially constant for the 103 days of exposure. Bloods drawn on the first day postexposure revealed that there was essentially no carboxyhemoglobin left and one month later the other hematological parameters had returned to baseline levels.

TABLE III  
HEMATOLOGICAL CHANGES\*

	<u>Date</u>	<u>Rbc x 10<sup>6</sup></u>	<u>Hemoglobin (gm %)</u>	<u>Hematocrit (Vol %)</u>	<u>Carboxyhemoglobin (% Sat)</u>
40 days Ambient	23 Jan	5.89 ± 0.48	13.5 ± 0.9	41 ± 3	--
	25 Feb	5.89 ± 0.30	12.1 ± 0.7	39 ± 2.5	--
	4 Mar	5.59 ± 0.26	11.3 ± 0.8	36 ± 3	--
	14 Mar	5.48 ± 0.27	11.2 ± 0.9	37 ± 3	--
28 days Alt	21 Mar	5.36 ± 0.38	11.3 ± 1.2	36 ± 4	--
103 days Alt + CO	22 Apr	6.25 ± 0.49	13.1 ± 1.1	43 ± 4	20.7 ± 2.2
	22 May	6.43 ± 0.47	14.2 ± 1.1	46 ± 4	22.1 ± 1.2
	1 Jul	5.99 ± 0.53	14.5 ± 1.1	46 ± 3	21.6 ± 1.4
30 days Ambient	24 Jul	5.66 ± 0.59	13.6 ± 1.3	44 ± 4	0.95±0.17
	19 Aug	4.96 ± 0.41	11.6 ± 0.9	39 ± 3	--

\*Means ± standard deviation

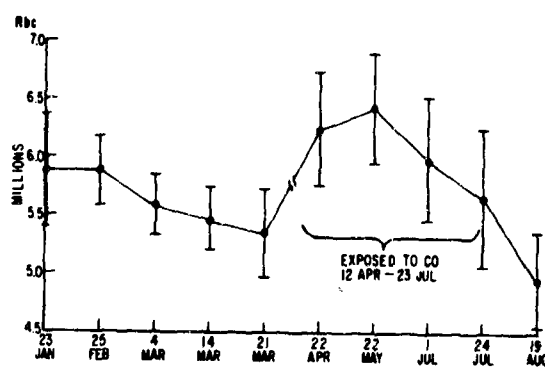


Figure 1. THE EFFECTS OF CARBON MONOXIDE ON RED BLOOD CELL COUNTS

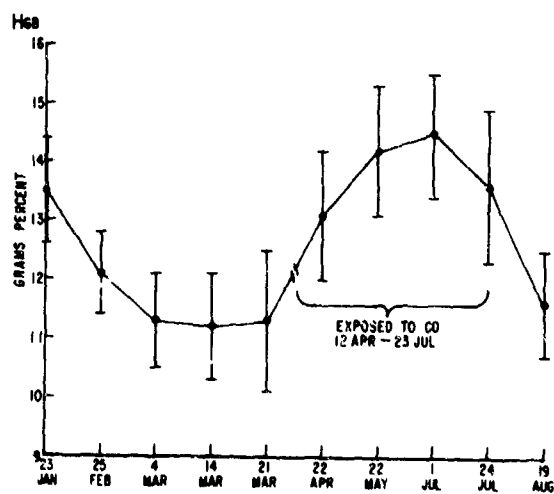


Figure 2. THE EFFECTS OF CARBON MONOXIDE ON HEMOGLOBIN LEVELS



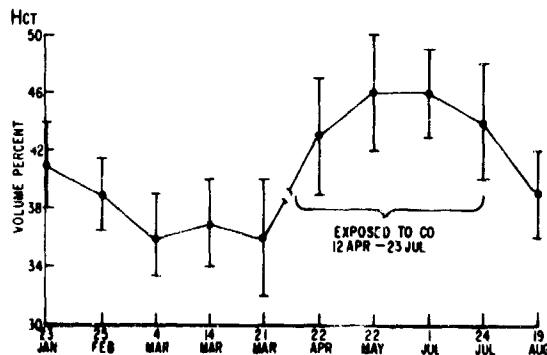


Figure 3. THE EFFECTS OF CARBON MONOXIDE ON HEMATOCRITS

Selected performance data are depicted in figure 4. These are the data from all 12 animals and indicate that performance was not significantly changed from control values during exposure. None of the animals, individually, showed any marked changes either clinically or in terms of performance. Other than the hematological changes mentioned before, none of the other clinical laboratory tests was significantly different from control values.

Despite mostly negative data from these monkeys, one animal became acutely ill 4 days following exposure and was killed using pentobarbital. The animal showed marked loss of appetite immediately before onset and was obviously dehydrated with chronic convulsions occurring before euthanasia. The pathology report could not establish the definite cause of illness but the microscopic changes were all compatible with the convulsive episodes. The diagnosis was idiopathic hypoglycemia with all other clinical laboratory tests being in the normal range. The death was not thought to be due to exposure to CO.

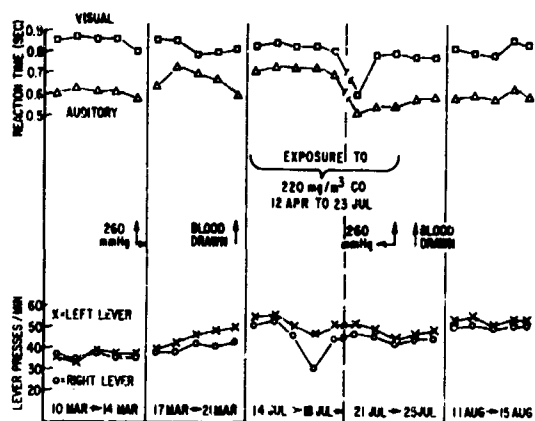


Figure 4. THE EFFECTS OF CARBON MONOXIDE ON PERFORMANCE IN 12 TRAINED MONKEYS

#### SUMMARY AND CONCLUSIONS

In order to test performance changes in animals, a continuous 100-day CO (220 mg/M<sup>3</sup> concentration) exposure study in a space cabin atmosphere of 5 psia total pressure (27,000 ft simulated altitude), mixed gas environment consisting of 68% oxygen and 32% nitrogen (pO<sub>2</sub> = 160 mm Hg) was performed. The experiment was performed on 12 trained *Macaca mulatta* monkeys in which operant behavior was conditioned to both continuous and discrete avoidance tasks by both audio and visual cues. The animals performed 15 min/hour, 8 hours/day, and 5 day/week, during the continuous exposure period. Extensive clinical laboratory determinations were performed, including blood carboxyhemoglobin levels, at regular intervals before, during, and following the exposure period. Results of the 100-day exposure indicated a rapidly plateauing carboxyhemoglobin saturation of approximately 22% in all animals. There was also a concomitant increase in hematocrit and hemoglobin. Exposure caused no detectable performance changes in any of the monkeys tested.

#### ACKNOWLEDGEMENTS

The invaluable technical assistance of SMSgt. John L. Naylor, Jr. and his staff of research chamber technicians is gratefully acknowledged. In addition, I am indebted to members of SysMed Corporation for carboxyhemoglobin determinations, and members of the Pathology Branch, especially Major W.F. MacKenzie, USAF, VC and MSgt. W.F. Hunt, Jr., for pathological evaluations and clinical chemistry.

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## DISCUSSION

LT. COLONEL STEINBERG (U.S. Army Environmental Hygiene Agency): Dr. Back, when you got to this 22% carboxyhemoglobin level, was anybody measuring anything like hypertension, cardiac rate, or anything like this?

DR. BACK: The answer is no. Not on these animals. However, Capt. Chikos is presently doing this work in another dome going at 440 milligrams per cubic meter of carbon monoxide in which we have a "zoo", but two of the dogs in there have been tested and there is no change in at least blood pressure and EKG after 3 months at 440 milligrams per cubic meter, and off the top of my head, I think the hematocrits on these animals are up around 60 or 61. The hemoglobins are over 20, so they have frank sludge running around in their blood vessels, but as far as I can tell, with no change in blood pressure or electrocardiogram. I think that answers it at this moment, but it's only two animals out of the 8 dogs that we tested. This takes some doing. The animals have to be anesthetized at altitude and all of the data monitored outside. We do have a monkey with implanted brain electrodes in that dome and we will be doing EEG's on this animal.

DR. SLONIM (Aerospace Medical Research Laboratory): Are you ruling out absolutely that there is no counter effect of the oxygen on the CO effect on performance in your monkeys?

DR. BACK: The 68% oxygen, if you remember, is at 260 millimeters of mercury, so the  $pO_2$  is exactly like it is in this room. This is not increased oxygen environment.

DR. WEIBEL (University of Berne): You may have said it, but it appears that the increase in hemoglobin just about compensates for the carboxyhemoglobin formed. Now, what is the half life of red cells in these monkeys? It seems to drop rather rapidly.

DR. BACK: I don't know what it is. I haven't the vaguest notion.

DR. WEIBEL: It seems to be rather rapidly going down.

DR. BACK: No, you have to look at the dates very closely. That drop was almost a month.

DR. WEIBEL: Still that's--

DR. BACK: Well, that's relatively rapidly. It's almost a month. That's why I had to bring them down. I kept getting bloods on them until I figured they were at preexposure levels before I went back up to 440, and because I didn't know where to stop, really, I had to pick a hemoglobin and hematocrit very close to what it was before. They are variable. The other thing is we are not sure what iron does here. We had to give our animals Chocks<sup>®</sup>, and so the animals were fed a vitamin with iron added, and hemoglobin still didn't increase. You see our preexposure hemoglobins were bothering us because when they were in the animal house they were up around 13.5 and by the time we started they were down around 11.5. We started to think: "Well, maybe they're losing blood, or not eating correctly", and we tried to compensate for that. We even gave them an injection of iron and that didn't seem to help them either.

DR. WEIBEL: Well, did you check the CO level in your animal house? Were your caretakers smoking all the time in there?

DR. BACK: No, they are not. They have to wear masks in that particular room.

DR. LEON (NASA, Ames Research Center): During that period, when the hematocrit and hemoglobin went down, were they in training then?

DR. BACK: As soon as they went in the domes they were in training, yes.

DR. LEON: Were they inactive, fairly inactive? Weren't running around?

DR. BACK: As you will see this afternoon, they are in a cage which is closed on three sides and they can only see out in front of them. They are relatively inactive. Although some of the animals appear to jump around, some of them don't. They're just sedentary.

DR. LEON: There's the decrease in red cell mass which accompanies bedrest and hypodynamia in general. This might be the cause.

DR. BACK: These are not that motionless, believe me. When we bleed them, they give us a good tussle.

DR. BENJAMIN: Is your increase in hemoglobin an increase of number of cells or an increase of the size of cells?

DR. BACK: The RBC's didn't change too much. Total numbers didn't go up too much. I don't think they were macrocytic or microcytic.

QUESTION: Could you give us more information on the type of tasks you used?

DR. BACK: Yes. I said each animal must press a left and right lever continuously every 15 seconds at least once or he's shocked, and he must push a button on audio

cue when a 2400 cps tone comes on within two seconds or he is shocked, and a white light comes on at random again, behind another button, and he must push it out within two seconds or he is shocked. Now, he gets 12 presentations, audio and visual, each 15 minutes, and the program goes on at eight in the morning, goes off at 8:15; goes on at 9:00, off at 9:15, and so forth until 3:15, at which time the chamber technician goes in, feeds and cleans them, et cetera. This is all published in the last proceedings. I didn't bother to go into the details.

DR. ALBRIGHT (Lockheed Missiles & Space Company, Sunnyvale): I have difficulty understanding what you consider your baseline hemoglobin in this experiment, and I would suggest that a cleaner experiment would be to have your sea level control in the dome to start with and wait until you have a baseline hemoglobin before you go to experimental variable.

DR. BACK: We did that, if you will recognize that they were on the ground - at a fairly stable level. That's from February to March, which is a one month period. I think you can see that there's no significant difference. They're all within one standard deviation.

DR. ALBRIGHT: How do you explain the first point?

DR. BACK: I said these animals were up in the animal house. Even that is not significantly different, but there is a trend. All I'm showing is a trend, and it's pretty obvious from this and other experiments that this is what's happening and you don't have to go to extensive statistical analysis to show that the increase in hemoglobin during CO exposure is really compensation. It has to be real.

## CARBON DIOXIDE TOLERANCE LEVELS FOR SPACE CABINS

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### INTRODUCTION

Current methods for maintaining the partial pressure of  $\text{CO}_2$  at levels less than 5 mm Hg in spacecraft cabin atmospheres have proved to be very successful in orbital and lunar missions to date. Despite the high degree of reliability for  $\text{CO}_2$  removal systems,  $\text{CO}_2$  contamination of a confined cabin atmosphere remains a realistic, potential problem in spaceflight operations. Although numerous experiments have been performed to determine the physiologic effects of  $\text{CO}_2$  on man, a delineation of safe exposure levels has not been well-established. Therefore, a series of related experiments was conducted in an attempt to define more precisely the tolerance levels for human exposure to elevated partial pressures of  $\text{CO}_2$ . The problem was approached with two primary goals: first, to determine the rate and degree of acclimatization of the resting man to elevated  $\text{CO}_2$ ; and second, to determine the physiologic effects of the added stress of exercise on tolerance to hypercapnia. This paper will review the findings of three resting chronic  $\text{CO}_2$  studies and two exercise hypercapnia studies, all of which were performed within the past two years.

### SUMMARY OF HYPERCAPNIA STUDIES

Table I describes the three chronic  $\text{CO}_2$  studies and two exercise-hypercapnia studies. In the first chronic experiment, 4 subjects were exposed to an ambient  $\text{P}_{\text{CO}_2}$  of 30 mm Hg for 11 days; in the second experiment, 4 different subjects were exposed to the same ambient  $\text{P}_{\text{CO}_2}$  for 5 days; and the third experiment consisted of a 30-day exposure of 4 additional subjects to an ambient  $\text{P}_{\text{CO}_2}$  of 21 mm Hg. Each experiment was designed to obtain repeated measurements of ventilation and acid-base indices of arterial blood and lumbar cerebrospinal fluid (CSF) during exposure to hypercapnia.

TABLE I

CHRONIC HYPERCAPNIA STUDIES			
SUBJECTS	AMBIENT $P_{CO_2}$ (mm Hg)	NUMBER OF DAYS	
4	30	11	
4	30	5	
4	21	30	
EXERCISE DURING EXPOSURE TO HYPERCAPNIA			
SUBJECTS	AMBIENT $P_{CO_2}$ (mm Hg)	WORKLOAD	DURATION (min)
8	0, 15, 21, 30	M-H (max)*	30
4	21 (acute-chronic)	L-M-H	45

\*0 and 21 mm Hg only - 22 minutes

In the first exercise-hypercapnia study, 8 subjects performed 30-minute periods of steady-state moderate exercise at 1/2 of their maximum oxygen consumption ( $\dot{V}O_2$ ) with inspired  $P_{CO_2}$  ( $P_{iCO_2}$ ) levels of "0", 8, 15 and 21 mm Hg, and heavy exercise (2/3 of maximum  $\dot{V}O_2$ ) at the same levels of  $P_{iCO_2}$  plus 30 mm Hg. The same subjects also performed a progressive exercise test to exhaustion (maximum  $\dot{V}O_2$ ) with  $P_{iCO_2}$  levels of "0", 8 and 21 mm Hg. Inspired  $P_{CO_2}$  closely approximates the ambient  $P_{CO_2}$  and, therefore, the two terms will be used interchangeably in this paper. In the second study, four additional subjects performed three levels of work (low, moderate, heavy) in air and after 1-hour of acute exposure and 15-20 days of chronic exposure to 21 mm Hg ambient  $P_{CO_2}$ . A 45-minute period of continuous steady-state exercise was performed at each work load. Measurements of minute ventilation, gas exchange and arterial  $P_{CO_2}$  ( $P_{aCO_2}$ ) and pH, along with electrocardiographic monitoring, received primary emphasis in the exercise tests which were performed on a bicycle ergometer. In both studies, the work load classified as heavy (H) was nearly identical in terms of heart rate (HR) and oxygen consumption ( $\dot{V}O_2$ ). Therefore, the physiologic responses to heavy exercise performed in air and in 21 mm Hg  $P_{iCO_2}$  may be compared in the two experiments.

## RESULTS OF THE CHRONIC HYPERCAPNIA STUDIES

### Ventilation, $P_{aCO_2}$ , CSF $P_{CO_2}$

Effects of the 5-day and 11-day exposures to an ambient  $P_{CO_2}$  of 30 mm Hg on minute ventilation ( $\dot{V}_E$ ),  $P_{aCO_2}$  and CSF  $P_{CO_2}$  are shown superimposed in figure 1. The shaded areas represent the data from the 5-day study. The exposure periods were preceded by 3 to 5-day control periods and followed by 2 to 5-day recovery



periods. In general, the results of  $\dot{V}_E$ ,  $\text{PaCO}_2$  and CSF  $\text{PCO}_2$  in the two studies paralleled each other very closely. Average ventilation increased abruptly at the start of  $\text{CO}_2$  breathing and then decreased slightly after the first day to remain essentially constant for the remainder of the exposure periods. During the recovery periods, the  $\dot{V}_E$  returned promptly to normal. After an initial elevation of about 2-4 mm Hg,  $\text{PaCO}_2$  increased an additional 1-3 mm Hg during the first day of hypercapnia and was not changed after 5 days in both studies, and after 9 days in the 11-day study. During the recovery periods, the  $\text{PaCO}_2$  had returned to normal when measured after 2-4 days. Average values of CSF  $\text{PCO}_2$  appeared to parallel those in arterial blood and the CSF  $\text{PCO}_2$  measured after one day of exposure was elevated by about 6 mm Hg in both studies and remained constant throughout the exposure periods.

Because the results of the 11-day study indicated that respiratory acclimatization to hypercapnia was essentially complete after one day of exposure, the first 24 hours of hypercapnia and the first 24 hours of recovery were studied more carefully in the 5-day experiment. Ventilation and  $\text{PaCO}_2$  were measured simultaneously at 4-hour intervals during the first day of exposure and the first day of recovery. Although not shown, it is interesting that the peak elevations of  $\text{PaCO}_2$  which were observed after 16 and 20 hours of exposure to an ambient  $\text{PCO}_2$  of 30 mm Hg and again after 16 and 20 hours of recovery were also observed at the same times during and after the 30-day exposure to an ambient  $\text{PCO}_2$  of 21 mm Hg. In all cases, the increases in  $\text{PaCO}_2$  occurred at the time of day when the subjects normally would have been sleeping. These data are indicative of a diurnal variation in the ventilatory response to  $\text{CO}_2$  similar to that reported by Egar et al (1968) and Koepchen et al (1953). It is possible that diurnal variation of hormone blood levels as shown by Von Euler (1955) may be partly responsible for the cyclic changes in ventilation and  $\text{PaCO}_2$ . The data summarized in figures 1, 2 and 3, except during the first 24 hours of exposure and recovery, were all obtained within a few hours after the subjects were awakened and should not be influenced by diurnal variation.

#### Arterial and CSF pH and Bicarbonate $[\text{HCO}_3^-]$

Average arterial and CSF pH and  $[\text{HCO}_3^-]$  data from the 5-day, 30 mm Hg experiment are summarized in figure 2. The arterial pH decreased acutely by 0.025 units, reached its lowest level at 20 hours of exposure, and then increased progressively until it returned to normal by the 5th day of exposure. Average CSF pH was lowest after 8 hours of exposure and then increased to a level very near the control value by the end of the exposure. Both arterial and CSF  $[\text{HCO}_3^-]$  increased progressively in parallel fashion to reach their maximum elevations by the 5th day of exposure. During the recovery period, all parameters returned to normal with the exception of CSF pH, which was lower than the control value in conjunction with slightly elevated CSF  $\text{PCO}_2$  after 2 days of recovery (see figure 1).

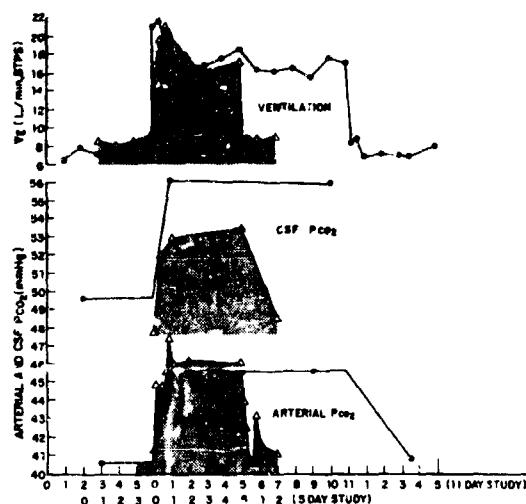


Figure 1. EFFECT OF A 5-DAY AND 11-DAY EXPOSURE TO AN AMBIENT  $P_{CO_2}$  OF 30 MM HG ON VENTILATION, ARTERIAL  $P_{CO_2}$ . The ambient oxygen concentration was maintained at about 19%-20% throughout the experiments; ambient  $P_{CO_2}$  was less than 2 mm Hg during the control and recovery periods. The data, which represent the average results of 3-4 subjects, were obtained from the unpublished observations of Menn et al (1968) for the 11-day study and Clark et al (1969) for the 5-day experiment.

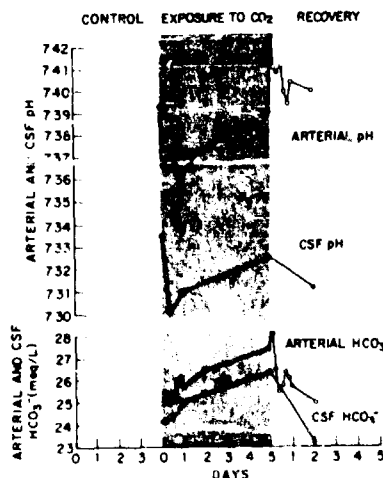


Figure 2. EFFECT OF A 5-DAY EXPOSURE TO AN AMBIENT  $P_{CO_2}$  OF 30 MM HG ON pH AND  $[HCO_3^-]$  OF ARTERIAL BLOOD AND CSF. Data were obtained from the same 5-day experiment described in figure 1.

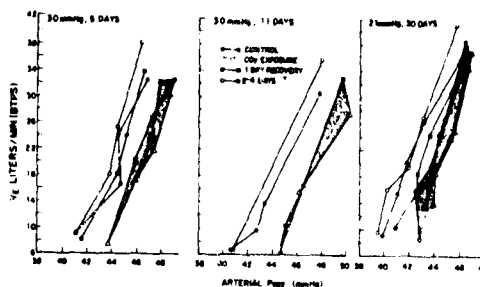


Figure 3. EFFECT OF CHRONIC EXPOSURE TO INCREASED AMBIENT  $P_{CO_2}$  ON THE RELATIONSHIP OF VENTILATION TO ARTERIAL  $P_{CO_2}$  ( $CO_2$ -RESPONSE CURVES). Data for the 5-day and 11-day studies were obtained from the same experiments described in figure 1, and from the observations of Clark et al (1969) for the 21 mm Hg, 30-day experiment.

#### Arterial $P_{CO_2}$ -Ventilation Response Curves

Data from the two 30 mm Hg  $P_{CO_2}$  experiments and the 21 mm Hg, 30-day experiment which show the relationship of ventilation to  $P_{CO_2}$ -ventilation response curves performed after at least 24 hours of exposure had shifted to the right of the control curve. These curves, along with other  $P_{CO_2}$ -ventilation response curves obtained at intervals during the exposure periods, were tightly grouped and are represented by the shaded areas in figure 3. Successive exposure curves indicated that there may have been a slight additional shift to the right of the 24-hour curve which was probably the result of the slow steady rise in arterial and CSF  $[HCO_3^-]$  (figure 2). After 24 hours of air breathing, the  $P_{CO_2}$ -ventilation response curves shifted back to the left toward the control curve and appeared to shift still further to the left after 2-4 days of recovery. Although the shifts in the  $P_{CO_2}$ -ventilation response curves appear to be small in magnitude, comparison of the ventilatory responses obtained at a constant  $P_{CO_2}$  show that ventilation after 24 hours of exposure to hypercapnia was considerably less than the ventilatory response to the same  $P_{CO_2}$  during the control and recovery periods. Schaefer (1949) has reported a similar shift to the right of the alveolar  $P_{CO_2}$ -ventilation response curves in normal men exposed to chronic hypercapnia, and Katsaros, et al (1960) produced the same result by acute administration of bicarbonate.

## RESULTS OF EXERCISE-HYPERCAPNIA STUDIES

Figures 4 (A, B), 5 (A, B) and 6 (A, B) illustrate, respectively, the minute ventilation ( $\dot{V}_E$ ),  $\text{CO}_2$  elimination ( $\dot{V}_{\text{CO}_2}$ ) and  $\text{PaCO}_2$  results of the two exercise-hypercapnia studies. Part (A) of each figure shows data from the study of graded exercise during acute and chronic exposure to 21 mm Hg ambient  $\text{P}_{\text{CO}_2}$ . The data are plotted against the mean heart rate (HR) responses to exercise rather than actual work load. Part (B) of each figure illustrates the results of a constant steady-state work load (2/3 of maximum  $\dot{V}_{\text{O}_2}$ ) performed in graded levels of hypercapnia with the data plotted against the inspired  $\text{P}_{\text{CO}_2}$ .

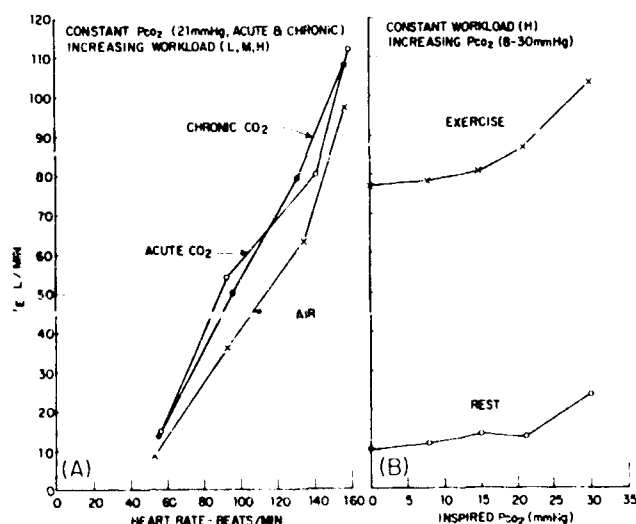
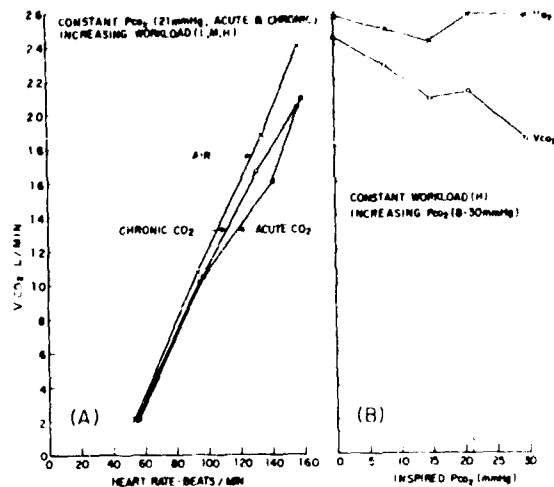


Figure 4. **EFFECT OF INCREASED AMBIENT  $\text{P}_{\text{CO}_2}$  ON RESPIRATORY MINUTE VOLUME DURING EXERCISE.** Part (A) shows the average results of 4 subjects during graded exercise in air and after acute (1 hour) and chronic (15-20 days) exposure to 21 mm Hg ambient  $\text{P}_{\text{CO}_2}$ , Sinclair et al (1969b). Mean heart rate responses to exercise were essentially the same at each work load for all experimental conditions and were used as an index of work effort. Part (B) represents the average results of 8 subjects during the performance of a heavy work load in graded levels of inspired  $\text{P}_{\text{CO}_2}$ , Menn et al (1968).



### Ventilation ( $V_E$ )

Figure 4 (A) shows that the average magnitude of  $V_E$  at similar work levels was essentially equal in acute and chronic hypercapnia with the air curve approximately 15 L/Min lower at each work load. Part (B) of figure 4 illustrates the increase in  $V_E$  in going from rest to exercise and shows that it was essentially equal with the same work load performed in lower levels of  $P_{iCO_2}$  (up to 15 mm Hg), but widened slightly at the higher levels of  $P_{iCO_2}$ . The data indicate that the increase in  $V_E$  with exercise in hypercapnia approximates a simple addition of increments with the possible exception at higher levels of  $P_{iCO_2}$  (21 and 30 mm Hg) where the effects of the combined stressors on  $V_E$  may be somewhat synergistic. These findings agree, in general, with those of Craig (1955) and Asmussen (1956). The changes in  $V_E$  with exercise in hypercapnia were brought about by increases in both respiratory rate and tidal volume. The latter contributed more to increasing  $V_E$  during exercise at a constant work load performed in progressive  $P_{iCO_2}$ , while changes in respiratory rate were more important in increasing the  $V_E$  with graded exercise performed in a constant ambient  $P_{CO_2}$ .

### Carbon Dioxide Elimination ( $V_{CO_2}$ )

Data showing the effect of exercise in hypercapnia on  $V_{CO_2}$  for the two studies are summarized in figure 5. In the first experiment, figure 5 (A) increases in  $CO_2$  elimination were progressive with increasing work load for all three experimental conditions; however, the curves were shown to be significantly different with the  $V_{CO_2}$  curves for acute and chronic hypercapnia falling below the air curve. The finding of decreased  $V_{CO_2}$  has been observed in other exercise-hypercapnia studies by Finkelstein (1968) and Sinclair et al (1969a). Part (B) of figure 5 shows that  $V_{CO_2}$  decreased significantly from 2.43 L/Min (STPD) to 1.84 L/Min over the "0" to 30 mm Hg  $P_{iCO_2}$  range with a constant work load. The fall in  $V_{CO_2}$  with exercise in hypercapnia has been shown not to be the result of reduced oxygen consumption as exemplified by the accompanying results for  $V_{O_2}$  in figure 5 (B). Since the respiratory system is the primary route for  $CO_2$  excretion, a decrease in  $V_{CO_2}$  with exercise in hypercapnia must represent a failure of fully adequate ventilation. It is suggested that the presence of  $CO_2$  in the inspired air reduces the quantity of  $CO_2$  that can be eliminated in each liter of expired air and, therefore, decreases the efficiency of alveolar ventilation. Retention of  $CO_2$  can be expected to continue until mean pulmonary capillary  $P_{CO_2}$  and  $V_E$  increase sufficiently to restore equality between  $CO_2$  elimination and  $CO_2$  production.

### Arterial $P_{CO_2}$ and pH

Figure 6 (A) shows that the differences between the mean  $P_{aCO_2}$  during acute and chronic  $CO_2$  and the corresponding  $P_{aCO_2}$  values in air increased progressively with increasing work load. Figure 6 (B) illustrates that changes in  $P_{aCO_2}$  between exercise in air and exercise at each level of hypercapnia also increased progressively with  $P_{iCO_2}$ . It can be noted that there was an increase in  $P_{aCO_2}$  of approximately 10-12 mm Hg associated with heavy exercise performed in 21 mm Hg ambient  $P_{CO_2}$  in both studies. A rise in  $P_{aCO_2}$  in going from rest to exercise in air is not the usual finding

in the literature. Generally, no change in  $\text{PaCO}_2$  is reported for moderate exercise (Holmgren and Linderholm, 1958) and a decrease in  $\text{PaCO}_2$  with heavy exercise (Asmussen, 1965). The progressive increase in  $\text{PaCO}_2$  with exercise in hypercapnia observed in these studies can be explained, in part, by retention of  $\text{CO}_2$  as indicated by the associated decrease in  $\text{VCO}_2$  (figure 5).

The arterial pH decreased progressively with increasing work load in the same ambient  $\text{PCO}_2$ , or with exercise at the same work load in increasing  $\text{PiCO}_2$ . In both studies, the metabolic acidosis component of the observed change in arterial pH was found to be less during exercise in hypercapnia than during the same exercise work load performed in air.

### SUMMARY AND CONCLUSIONS

With the exception of occasional mild headaches and awareness of increased ventilation during the first 24 hours of hypercapnia, the subjects tolerated chronic exposure to 21 and 30 mm Hg ambient  $\text{PCO}_2$  without apparent difficulty. It is noteworthy that the investigators and technicians entered these hypercapnic atmospheres for periods of 2-8 hours on a daily basis. Very little difficulty was reported by this group other than the awareness of increased ventilation which was especially noticeable in the 30 mm Hg environments. The investigators did not find it difficult to perform physiological tests and measurements during these exposures and their awareness of increased ventilation appeared to subside 1-2 hours following the abrupt exposure.

The objective results of the chronic studies indicate that most of the reduction in the ventilatory response to  $\text{CO}_2$  occurs within the first 24 hours of exposure to hypercapnia even though the maximum compensation of the initial decrease in arterial pH requires up to 5 days. It is suggested that a central component of respiratory control which is relatively insensitive to changes in arterial pH is responsible for the early ventilatory acclimatization.

The data summarized in figures 4-6 indicate that some of the normal responses to exercise in air are significantly modified by the simultaneously imposed stress of exposure to atmospheres containing 8-30 mm Hg  $\text{PiCO}_2$ . Notable in this respect were elevation in  $\text{VE}$  and retention of  $\text{CO}_2$  as manifested by a decrease in  $\text{VCO}_2$  and increase in  $\text{PaCO}_2$ . Nine incidents of cardiac ectopic foci were recorded during the exercise studies; however, one subject was responsible for 5 of the ECG abnormalities and it was not possible to correlate the changes in ECG with work load or level of ambient  $\text{PCO}_2$ . In some cases, the ectopic foci were associated with exercise in the "0"  $\text{PiCO}_2$  environment.

Despite the observed physiologic changes, no difficulty was encountered by the exercising subjects at low  $\text{CO}_2$  levels of 8 and 15 mm Hg  $\text{PiCO}_2$ . Higher levels of hypercapnia (21 and 30 mm Hg) caused some symptoms of dyspnea and intercostal muscle pain, but were of mild enough degree to permit all subjects to complete their exercise runs. The tolerance to maximum exercise in 21 mm Hg resembled that to the heavy work load in 30 mm Hg  $\text{PiCO}_2$ . Physiologic responses to graded exercise during acute and chronic exposure to 21 mm Hg ambient  $\text{PCO}_2$  were found to be essentially the same.

It should be emphasized that these conclusions are based upon data obtained from relatively small groups of subjects. In addition, individual variations in both objective and subjective responses to  $\text{CO}_2$  inhalation were seen in the resting and exercising subjects. While the range of  $\text{CO}_2$  partial pressures employed in these studies appears to be acceptable for human exposure, more definitive information will be obtained by exposure of larger numbers of subjects to higher levels of hypercapnia and additional combinations of exercise and  $\text{PICO}_2$ . These studies are presently in the planning stages.

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## DISCUSSION

DR. BACK: Have you tried to prejudice your results by giving a carbonic anhydrase inhibitor?

MAJOR SINCLAIR (USAF School of Aerospace Medicine): No, we haven't. We are just interested in the objective responses of the subjects without tampering with the acid-base parameters in any way at this time.

MR. WANDS (National Academy of Sciences): Did you do any blood chemistries on these people? For example, calciums?

MAJOR SINCLAIR: No, we did not. These studies have been done in earlier chronic hypercapnia studies. The results of the calcium/phosphorus analytical tests in previous studies were negative. Other investigators have shown changes in calcium--phosphorus levels in the blood as well as the urine. In most cases these were chronic animal exposures to higher levels of hypercapnia. In our upcoming studies we plan to go to a higher ambient  $p\text{CO}_2$ , try 40 mm of mercury, if this appears to be feasible in pilot runs, and we do plan to get better calcium balance studies in this test.

DR. COULSTON (Albany Medical College): Just a question about the format of the experiment. How did you distinguish between the normal apprehensive response of these people when they knew they were going to get more and more of  $\text{CO}_2$  or something else? In other words, how did you double blind out the normal increase of heart rate and so on as the atmosphere was changed?

MAJOR SINCLAIR: Well, there was no secret about when the subjects were going to be exposed to the  $\text{CO}_2$ . All of our chronic studies were preceded by a suitable control period during which the subjects lived in the environmental chambers. They were moved only temporarily from the environmental chamber long enough to allow the environmental control system to bring the  $\text{CO}_2$  to its desired level, and then the subjects walked back into the chamber with an abrupt exposure to the ambient  $\text{CO}_2$ . They were well aware of the increase in the environment. We made no effort at all to hide this from them or to prevent these excitatory changes which might have been in existence. We really can't differentiate any effects purely from the atmosphere, such things as heart rate, from the effects of general excitement of the initial exposure. One thing we did not see, and I think this has been reported, especially by Dr. Karl Schaefer and in other chronic  $\text{CO}_2$  studies; there appears to be an initial period of excitation followed later, shortly later, by a period of depression. In our chronic exposures to hypercapnia we did not see these phases or waves in the state of excitation of the CNS. I think it's kind of hard, anyway, to say that you're seeing a state of

depression and not seeing just depression from being in the chamber, knowing you're going to be there an additional 15 days or what have you.

DR. BACK: I'm still wondering what's going on in the urine of these people. What about pH and  $p\text{CO}_2$  and bicarbonates and sodiums or potassiums? Did you do any urine work at all? You kept alluding to the fact that you say that you've got a decreased excretion as one of the mechanisms, and I'd like to know what's going on in the urine.

MAJOR SINCLAIR: We have included urinary studies in all of our chronic tests. We look at nitrates and acidity, and ammonia. In previous studies, we looked at calcium and phosphorus in the urine. We have been unable to see significant changes in these parameters in the levels of hypercapnia in chronic exposure we have used in our studies. I don't know whether this is the result of methodology or whether we are not seeing changes. We cannot pick them up in the urinary tests we have been using.

MAJOR THEODORE: I think that this was a pretty nice study as far as showing the chronic effects of hypercapnia. There was one point, maybe just a point of clarification, to explain your alveolar  $\text{CO}_2$  gradients. You sort of said this was due to a reduction in alveolar ventilation. I think you probably really meant this was a disturbance in VQ abnormalities. There's a tremendous amount of data on this point. It's just a moot point, actually. Alveolar ventilation did actually increase. Theoretically, your arterial  $p\text{CO}_2$  should equal alveolar  $p\text{CO}_2$  and the only way to get gradients of this type has to be through VQ abnormalities, since no diffusion barriers to  $\text{CO}_2$  have ever really been demonstrated. I think Cadell especially has used this type of  $\text{CO}_2$  gradient to pick up early vascular lesions in the lung, which is common not only with vasculitis but with embolic phenomena.

MAJOR SINCLAIR: Let me clarify one point. I was not speaking of alveolar arterial gradients. The difference I was speaking of was in relation to the arterial  $p\text{CO}_2$  with given exercise workload in hypercapnia as compared to the same arterial  $p\text{CO}_2$  response to the same workload performed in air. That difference is what I was looking at.

MAJOR THEODORE: I retract everything.

MAJOR SINCLAIR: All of our arterial blood sampling was done simultaneously with measurements of alveolar  $p\text{CO}_2$  and consistently we saw about a 2 mm gradient. We did not see a change. Also I was not speaking of alveolar ventilation; I was speaking of a decreased efficiency of alveolar ventilation to remove  $\text{CO}_2$  in hypercapnia environments.

MAJOR THEODORE: By definition that's what occurs. I thought when you were talking about your gradients you were really talking about  $\text{CO}_2$ . . . alveolar arterial gradients.

MAJOR SINCLAIR: Then I would have to agree with you that we would have a diffusion problem there.

DR. HODGE: What sort of space cabin CO<sub>2</sub> levels did you mention at the start? Had they all been maintained below some figure?

MAJOR SINCLAIR: To my knowledge it has been maintained below 5 mm of mercury. Possibly it has jumped up to 6, 7, or 8. I don't know, but from what I gather in all the flights to date, this is just in the space cabin, the pCO<sub>2</sub> has been maintained lower than 5 mm of mercury. I think in the extravehicular activity the breathing CO<sub>2</sub> pressure probably jumped up higher on occasions.

## THE EFFECT OF BROMOTRIFLUOROMETHANE ON OPERANT BEHAVIOR IN MONKEYS

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### INTRODUCTION

The Apollo and School of Aerospace Medicine fires in January of 1967 have stimulated a great deal of interest by the U.S. Air Force in the search for satisfactory fire suppression agents for use in oxygen rich atmospheres. Agents, including water and various haloalkanes, have been evaluated for effectiveness in these atmospheres (Botteri and Manheim, 1969). Of the agents for which some fire fighting experience exists, only water and bromotrifluoromethane ( $\text{CBrF}_3$ ) potentially fulfilled the established requirements, with  $\text{CBrF}_3$  being the most effective on a pound for pound basis.

The chemical similarity between these haloalkanes and other known central nervous system depressants, such as halothane, has necessitated comparative toxicological evaluation of these compounds. The importance of these evaluations is magnified greatly since the agent may be used in a closed environmental system such as a space cabin.  $\text{CBrF}_3$  has been shown to be least toxic of five haloalkane fire extinguishants tested in rats (Clayton, 1967). Paulet (1962) could not demonstrate any subjective alterations in rabbits, mice, guinea pigs, or rats when exposed to concentrations of less than 50%  $\text{CBrF}_3$ . This apparent lack of toxicity at effective fire suppressing levels increased the desirability of this compound even more.

Van Stee (1969a) reported lethargy and loss of aggressiveness in conscious monkeys exposed to 50-80%  $\text{CBrF}_3$ . Alterations in the electroencephalogram (EEG) of unanesthetized monkeys exposed to  $\text{CBrF}_3$  at concentrations of 50% and higher have also been observed (Van Stee, 1969b). The EEG patterns produced by  $\text{CBrF}_3$  differed from those produced by halothane in that tactile and visual stimuli could cause activation of the EEG during exposure to  $\text{CBrF}_3$ . Significantly lower concentrations have been shown

to induce varying degrees of performance decrement in human subjects (Hine, 1969). A majority of these subjects exhibited signs of beginning inebriation or analgesia at concentrations of  $\text{CBrF}_3$  between 10-15%. Hine indicated that loss of consciousness would have occurred in most subjects at concentrations between 15 and 25%  $\text{CBrF}_3$ .

The purpose of this investigation was to quantify any possible performance decrement expected on the basis of human exposures and to correlate these with changes seen in other animal data.

## METHODS

**APPARATUS.** An exposure chamber similar to the one utilized by Reynolds and Back (1966) was made practically airtight for this study (figure 1). The 15.5 inch square test panel mounted on one wall of the test chamber contained 2 response levers each with a stimulus light mounted 1 inch above a lever, and 2 push button switches. A stimulus light was mounted behind one push button, and a 1 kHz speaker was mounted behind the other push button. A plexiglas window in one side of the chamber was utilized for observation of the subject during exposure.

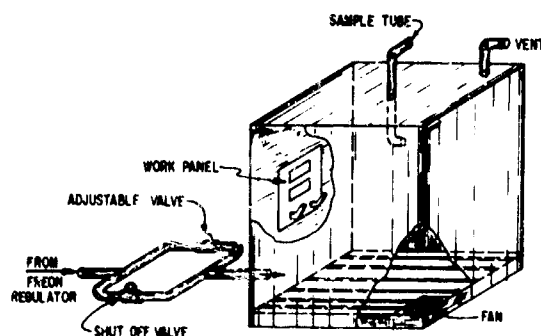


Figure 1. SCHEMATIC DIAGRAM OF EXPOSURE APPARATUS.

$\text{CBrF}_3$  (1) and oxygen were introduced into the chamber through a portal in the lower left wall containing the performance panel. A fan installed under the shock bars in the rear left side of the chamber directed the flow of gas upward toward the area occupied by the monkey in front of the performance panel. A vent in the upper right rear corner of the cage permitted a smooth flow of the freon from the bottom left side to the upper right side of the chamber. A line for obtaining chamber air samples was suspended in the middle of the chamber at approximately the monkey's breathing zone. Air samples for  $\text{CBrF}_3$  analysis obtained from lines placed at four other locations in the chamber did not differ more than 1% from that obtained from the primary sampling line. This indicates that adequate mixing of the  $\text{CBrF}_3$  and the chamber atmosphere was obtained.

The  $\text{CBrF}_3$  was admitted into the exposure chamber by means of two parallel lines which were joined as a common connection both at the tank regulator and at the chamber attachment. One line was equipped with a shut-off valve and the other with a needle valve which permitted a regulation of  $\text{CBrF}_3$  flow of 0-2 cu ft/min. Chamber  $\text{CBrF}_3$  concentrations were determined by use of a Model 154 Perkin-Elmer vapor fractometer. (2) Chamber oxygen partial pressure was monitored by a Beckman model 2 C gas analyzer. A console of electronic relay circuitry was used for programming the performance tasks and recording results.

**PERFORMANCE SCHEDULE.** The schedule of 15 minutes duration was the same as that used by Reynolds and Back (1966) with one modification and one addition. In addition to the "Dual Continuous Avoidance" and visual stimulus, an auditory stimulus consisting of a 1 kHz tone was added. The subject was required to turn off the stimulus by pressing the response key within 2 seconds (auditory response time, ART). Failure to respond within the allotted time resulted in a shock of the same magnitude as for the other tasks. Both auditory and visual stimuli were presented 30 seconds apart once each minute. This permitted a minute by minute recording of visual response time (VRT) and auditory response time (ART) as well as performance proficiency on the dual continuous levers (left and right lever presses, LLP and RLP).

**PROCEDURE.** Five male and two female adult monkeys (*Macaca mulatta*) weighing between 5.1 and 8.0 kg were selected for this study. All monkeys had been trained on the performance tasks to peak proficiency over a period of several months. Prior to any given exposure, the subject was placed in the exposure chamber and at least 6 fifteen minute work sessions were completed. A rest period of at least 15 minutes was allowed between work sessions. If the subject appeared to be performing at a stable level on all tasks, one 15 minute session was completed in which a minute by minute breakout of performance on each task was recorded. After a fifteen minute rest period, the chamber was purged with oxygen for 5 minutes which was sufficient to increase the

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(1) Freon 1301, E. I. duPont de Nemours & Co., Wilmington, Delaware.

(2) Perkin Elmer Corporation, Norwalk, Connecticut.

oxygen concentration above 90%. Upon completion of the oxygen purge, the oxygen was turned off and freon exposure started. At this point the fifteen minute work session was started with a minute by minute recording of performance.

$\text{CBrF}_3$  exposure was accomplished in the following steps. (1) Both the needle valve and the shut off valve were opened to allow maximum flow of the freon into the chamber. The time-concentration relationship for this maximum flow is presented in figure 2. (2) When sufficient time had elapsed with a maximum flow, the shut-off valve was closed and the needle valve turned back to a flow rate sufficient for maintaining approximately the peak concentration attained. (3) After 4 minutes of exposure at the peak concentration, the needle valve was closed and the oxygen purge started. The oxygen flow was sufficient to return the  $\text{CBrF}_3$  concentration to 1% or less in 1-5 minutes depending upon the end peak concentration of the freon.

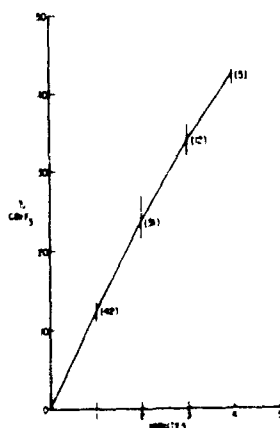


Figure 2. THE CONCENTRATION OF  $\text{CBrF}_3$  IN THE EXPOSURE CHAMBER DURING MAXIMUM FLOW. Vertical lines represent the range. Numbers in parenthesis represent the number of times each concentration was recorded.



The chamber environment was sampled every 1.5 minutes for  $\text{CBrF}_3$  analysis starting one minute after exposure was started. Oxygen partial pressures were recorded each minute after exposure was started. After the fifteen minute exposure work session, the subject was rested for 15 minutes and another 15 minute work session completed to determine any residual effects of exposure. No monkey was exposed to the  $\text{CBrF}_3$  more than once during any seven day period.

The 15 one minute baseline scores were paired with the 15 one minute exposure scores for statistical evaluation of performance on each task. Differences between the baseline and exposure scores were determined by Students t test at the 1% level of significance.

## RESULTS

A summary of the performance data is presented in table I. All subjects exhibited a decrement in performance on at least one task at  $\text{CBrF}_3$  concentrations of 20-25%. In general, increasing the concentration of  $\text{CBrF}_3$  in each individual subject results in loss of performance on an increasing number of tasks. An obvious exception to this graded concentration-response relationship can be observed in subject No. 3. A performance decrement on one of the continuous avoidance tasks (RLP) is seen at 22.5-25%  $\text{CBrF}_3$ . Although performance decrements appear in the discrete avoidance tasks at the higher concentrations, no more than one task shows a significant decrement at any given concentration.

TABLE I  
RESULTS OF  $\text{CBrF}_3$  EXPOSURE ON MONKEY PERFORMANCE

TABLE OF PERCENT	LAP	SLP	ART	VVT	TABLE OF PERCENT	LAP	SLP	ART	VVT
31.0-42.0	1	1	1	1	32.0-43.0	1	1	1	1
32.0-43.0	1	1	1	1	33.0-44.0	1	1	1	1
33.0-44.0	1	1	1	1	34.0-45.0	1	1	1	1
34.0-45.0	1	1	1	1	35.0-46.0	1	1	1	1
35.0-46.0	1	1	1	1	36.0-47.0	1	1	1	1
36.0-47.0	1	1	1	1	37.0-48.0	1	1	1	1
37.0-48.0	1	1	1	1	38.0-49.0	1	1	1	1
38.0-49.0	1	1	1	1	39.0-50.0	1	1	1	1
39.0-50.0	1	1	1	1	40.0-51.0	1	1	1	1
40.0-51.0	1	1	1	1	41.0-52.0	1	1	1	1
41.0-52.0	1	1	1	1	42.0-53.0	1	1	1	1
42.0-53.0	1	1	1	1	43.0-54.0	1	1	1	1
43.0-54.0	1	1	1	1	44.0-55.0	1	1	1	1
44.0-55.0	1	1	1	1	45.0-56.0	1	1	1	1
45.0-56.0	1	1	1	1	46.0-57.0	1	1	1	1
46.0-57.0	1	1	1	1	47.0-58.0	1	1	1	1
47.0-58.0	1	1	1	1	48.0-59.0	1	1	1	1
48.0-59.0	1	1	1	1	49.0-60.0	1	1	1	1
49.0-60.0	1	1	1	1	50.0-61.0	1	1	1	1
50.0-61.0	1	1	1	1	51.0-62.0	1	1	1	1
51.0-62.0	1	1	1	1	52.0-63.0	1	1	1	1
52.0-63.0	1	1	1	1	53.0-64.0	1	1	1	1
53.0-64.0	1	1	1	1	54.0-65.0	1	1	1	1
54.0-65.0	1	1	1	1	55.0-66.0	1	1	1	1
55.0-66.0	1	1	1	1	56.0-67.0	1	1	1	1
56.0-67.0	1	1	1	1	57.0-68.0	1	1	1	1
57.0-68.0	1	1	1	1	58.0-69.0	1	1	1	1
58.0-69.0	1	1	1	1	59.0-70.0	1	1	1	1
59.0-70.0	1	1	1	1	60.0-71.0	1	1	1	1
60.0-71.0	1	1	1	1	61.0-72.0	1	1	1	1
61.0-72.0	1	1	1	1	62.0-73.0	1	1	1	1
62.0-73.0	1	1	1	1	63.0-74.0	1	1	1	1
63.0-74.0	1	1	1	1	64.0-75.0	1	1	1	1
64.0-75.0	1	1	1	1	65.0-76.0	1	1	1	1
65.0-76.0	1	1	1	1	66.0-77.0	1	1	1	1
66.0-77.0	1	1	1	1	67.0-78.0	1	1	1	1
67.0-78.0	1	1	1	1	68.0-79.0	1	1	1	1
68.0-79.0	1	1	1	1	69.0-80.0	1	1	1	1
69.0-80.0	1	1	1	1	70.0-81.0	1	1	1	1
70.0-81.0	1	1	1	1	71.0-82.0	1	1	1	1
71.0-82.0	1	1	1	1	72.0-83.0	1	1	1	1
72.0-83.0	1	1	1	1	73.0-84.0	1	1	1	1
73.0-84.0	1	1	1	1	74.0-85.0	1	1	1	1
74.0-85.0	1	1	1	1	75.0-86.0	1	1	1	1
75.0-86.0	1	1	1	1	76.0-87.0	1	1	1	1
76.0-87.0	1	1	1	1	77.0-88.0	1	1	1	1
77.0-88.0	1	1	1	1	78.0-89.0	1	1	1	1
78.0-89.0	1	1	1	1	79.0-90.0	1	1	1	1
79.0-90.0	1	1	1	1	80.0-91.0	1	1	1	1
80.0-91.0	1	1	1	1	81.0-92.0	1	1	1	1
81.0-92.0	1	1	1	1	82.0-93.0	1	1	1	1
82.0-93.0	1	1	1	1	83.0-94.0	1	1	1	1
83.0-94.0	1	1	1	1	84.0-95.0	1	1	1	1
84.0-95.0	1	1	1	1	85.0-96.0	1	1	1	1
85.0-96.0	1	1	1	1	86.0-97.0	1	1	1	1
86.0-97.0	1	1	1	1	87.0-98.0	1	1	1	1
87.0-98.0	1	1	1	1	88.0-99.0	1	1	1	1
88.0-99.0	1	1	1	1	89.0-100.0	1	1	1	1
89.0-100.0	1	1	1	1					
90.0-100.0	1	1	1	1					
91.0-100.0	1	1	1	1					
92.0-100.0	1	1	1	1					
93.0-100.0	1	1	1	1					
94.0-100.0	1	1	1	1					
95.0-100.0	1	1	1	1					
96.0-100.0	1	1	1	1					
97.0-100.0	1	1	1	1					
98.0-100.0	1	1	1	1					
99.0-100.0	1	1	1	1					
100.0-100.0	1	1	1	1					

Human Numerals Designate Subject Numbers  
 1 = Performance Decreased Significantly at P < 0.01 (one-tailed test)  
 LRP = Left Lower Pressure      ART = Auditory Response Time  
 SLP = Right Lower Pressure      VVT = Visual Response Time

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Overall discrete avoidance (DA) performance for each 15 minute work session was obtained by combining the total auditory and visual response times. Similar treatment of right and left lever presses provided total continuous avoidance (CA) performance for each session. The effect of increasing  $\text{CBrF}_3$  concentrations on total CA and DA for the entire group was then determined by pairing preexposure and exposure work sessions. The mean total response times (DA) for the 7 monkeys increased significantly at  $\text{CBrF}_3$  concentrations from 20-30% and higher (figure 3). Although all monkeys experienced a performance decrement on at least one continuous avoidance task (RLP or LLP) during exposure to the  $\text{CBrF}_3$ , as seen in table 1, the mean total continuous avoidance (CA) performance for all the monkeys was not significantly altered (figure 4).

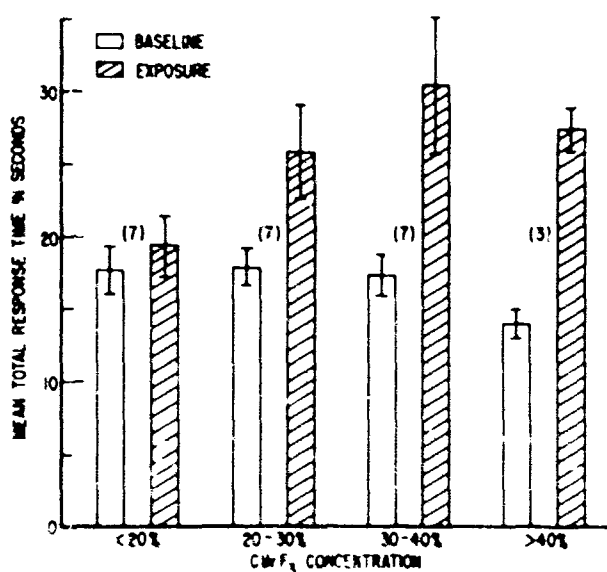


Figure 3. EFFECT OF  $\text{CBrF}_3$  ON MEAN TOTAL RESPONSE TIME FOR A 15-MINUTE WORK SESSION. Open bars represent mean pre-exposure values and hatched bars represent exposure values. I shaped bars represent standard error of the mean. Numbers in parenthesis indicate number of monkeys exposed in that concentration range.

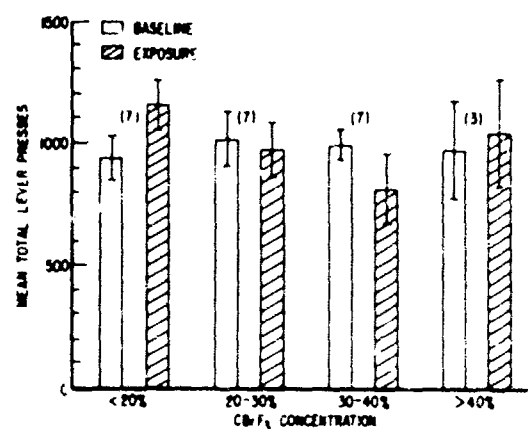


Figure 4. EFFECT OF CBrF<sub>3</sub> ON MEAN TOTAL LEVER PRESSES FOR A 15-MINUTE WORK SESSION. Open bars represent mean preexposure values and hatched bars represent exposure values. I shaped bars represent standard error of the mean. Numbers in parenthesis indicate number of monkeys exposed in that concentration range.

At the higher concentrations, some of the subjects completely ceased performing during the period of peak exposure. This was not accompanied by any visible depression as expected. On the contrary, the subjects were very alert. They attempted to escape by trying to climb the cage walls or by holding on to the sampling tube suspended from the top of the cage. Once the CBrF<sub>3</sub> concentration was reduced, they would immediately return to the performance panel and start working.

Recovery from the effects of CBrF<sub>3</sub> exposure was very rapid. All subjects returned to normal levels of performance within 1-3 minutes after the freon concentration was reduced to 5% or less. There was no significant decrement in performance noted in any of the fifteen minute work sessions immediately following the exposure period.

#### DISCUSSION

The experimental design may have prevented demonstration of additional statistically significant decrements in the continuous avoidance tasks (RLP and LLP) at the intermediate and lower levels of CBrF<sub>3</sub>. The subjects available for testing had been trained on fifteen minute work sessions. The short exposure periods necessary for observing the rate of recovery from exposure provided relatively few data points during

peak exposure. A major disadvantage of this design is evident in figure 5. The onset of performance decrement to the point of complete impairment and the rapid recovery are visibly related to the  $\text{CBrF}_3$  concentration. However, the subject's overcompensation resulting in marked increased lever presses during the last 6 minutes of the postexposure period prevented the demonstration of a statistically significant decrement for the entire 15 minute work session. Had the experiment been designed to maintain a peak exposure for a longer duration of the 15 minute test period, it is quite probable that performance decrement would have been observed in more continuous avoidance tasks at the intermediate and lower levels of exposure. This tendency to overcompensate on the continuous avoidance tasks is also responsible for the apparent discrepancy between the continuous avoidance data when analyzed individually (table I) and when expressed as mean total lever presses (figure 4).

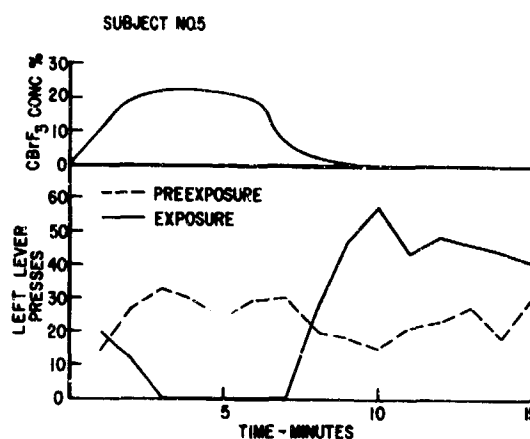


Figure 5. THE EFFECT OF 20-23%  $\text{CBrF}_3$  EXPOSURE ON THE NUMBER OF LEFT LEVER PRESSES IN SUBJECT NUMBER 5. Preexposure work session indicated by dotted line. Exposure work session represented by solid line.

The absence of any outwardly visible signs of central nervous system depression during  $\text{CBrF}_3$  exposure is of added importance. Even at concentrations high enough to cause complete loss of ability to perform on any of the conditioned tasks, the subjects still retained all faculties necessary to provide escape behavior. One subject succeeded in escaping the shocks by standing on the small fan in the corner of the cage while holding on to the sampling tube. Almost immediately after the  $\text{CBrF}_3$  concentration was reduced, the subject resumed performing as before, although he evidently could have

maintained his escape position for the duration of the 15 minute session. His action necessitated placing the fan under the shock grid. Attempts at various other less successful methods of escape were seen in the other subjects.

Van Stee (1969b) observed that tactile and visual stimuli could alter the EEG pattern in monkeys exposed to  $\text{CBrF}_3$  concentrations as high as 80%. Further investigation by Chikos (1969), using brain implanted electrodes, indicates that  $\text{CBrF}_3$  produced cortical depression with sparing of the reticular activating system. This would permit alterations in performance without loss of consciousness. These observations coupled with the results of this investigation suggest that the impairment in performance caused by  $\text{CBrF}_3$  cannot be attributed to the sedation and analgesia characteristics of such halogenated anesthetics as chloroform and halothane.

#### ACKNOWLEDGEMENTS

The authors wish to express their thanks to Technical Sergeant John R. Bruce for his technical assistance and to Mr. Stanley Erk and Mr. Lloyd Kaczmarek of the SysMed Corporation, Dayton, Ohio, for their assistance in assembling the apparatus.

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## DISCUSSION

MR. WANDS: Major, I'm not quite clear on the environment during the actual exposure time. Was this a hundred percent oxygen plus  $\text{CBrF}_3$ ?

MAJOR CARTER (Aerospace Medical Research Laboratory): At the beginning, there was an oxygen purge which brought the oxygen concentrations up to around 90%. Then we introduced the Freon where by necessity the Freon was replacing a certain amount of the oxygen. To answer your question, we did monitor oxygen concentration, oxygen partial pressures, which I didn't include. We did monitor the oxygen partial pressures each minute and they never fell below 50%. Since these were quite short exposure, this is one reason we didn't expose for a longer period of time, and if I had to do it over again I'd make my exposure much longer, but  $\text{CBrF}_3$  is expensive.

MR. WANDS: In a dynamic system you're pumping a lot out the stack.

MAJOR CARTER: Yes, you see a lot of dollars going out.

MR. WANDS: It's interesting that the monkeys were aware of this material.

MAJOR CARTER: This is something that I did have to fight a little bit. We would have one or two monkeys that would start expecting it, so in some of my previous runs I would hook up air and go through the whole thing so they would not start guessing, and when they hear the flow, they'd maybe start trying to get away from the shock. So I would do this frequently enough to, hopefully, confuse them. Of course, if they could smell it, that would be another thing and we couldn't get away from that.

COLONEL BELL (AF Surgeon General Office): Could you relate the concentrations and the times to the ability of  $\text{CBrF}_3$  to put out a fire in the oxygen environment?

MAJOR CARTER: We have the data from Mr. Botteri, and there are a large number of graphs, oxygen concentrations,  $\text{CBrF}_3$  concentrations and pressures. I believe that it requires around 50%  $\text{CBrF}_3$  to put out a fire at one atmosphere oxygen.

MAJOR VAN STEE (AFIT, Ohio State University): That is right.

MAJOR CARTER: It is an effective agent, even more effective than water, on a pound for pound basis.

COLONEL BELL: As long as you don't let the fire get away from you at the start.

DR. COULSTON: This was the same question that I was going to ask. Let me relate this now to air. What  $\text{CBrF}_3$  concentrations would you have to use if you were going to put out a fire in just ambient air?

MAJOR CARTER: It seems to be no problem. I think you can put out a fire in ambient air at something around 6%.

MR WANDS: Six percent is correct.

DR. COULSTON: Then I raise the question, did you do any studies below 20%?

MAJOR CARTER: Yes, sir.

DR. COULSTON: To know how much of a safety factor might be involved?

MAJOR CARTER: I wasn't testing for safety factor, as such. Once again, I'd like to point out this is a very robust task. Now, according to my psychologist friends, they say this is the type of task that right before you die you'll reach up and grab that lever, so you could theoretically have a decrement even with lower concentrations than this. I didn't answer your question.

DR. COULSTON: That's all right. That's close enough. I'm very intrigued.

MAJOR CARTER: We tested at levels around 15% on all the animals and got no decrement using this task.

DR. COULSTON: I was very intrigued about your conclusions that this compound is behaving differently than the halothane anesthetics. Do you care to expand on that a little bit?

MAJOR CARTER: I will. This is true. This study doesn't suggest a mechanism at all. I do suggest it is not the same mechanism as halothane, and Dr. Chikos, who follows me, will expand on this.

DR. COULSTON: I see. Thank you very much.

CAPTAIN SCHWARTZ (Aerospace Medical Research Laboratory): I was wondering about the decrement when you get about 4 or 5 of the tasks that have a decrement in them. How does the performance of the animals, visually, look at this stage?

MAJOR CARTER: This is the area of which I'm speaking, when the subject will really start trying to get away from the shock. I say it's getting away from the shock. My psychologists say I can't say he's getting away from the shock, but he's obviously doing something - racing around the cage, trying to grab onto the walls, the levers, anything else he can do. I think he knows that a shock is coming. I would.

CAPTAIN SCHWARTZ: Would you say it would be an inebriation effect in the beginning? This sounds as though he's either more hyper, more aware of what's happening. Or is it stimulation rather than inebriation?

MAJOR CARTER: I wouldn't say it's stimulation, because if you just put an animal in the cage in which he has nothing to do, he doesn't jump up and race around the cage. He just sits there. This is completely subjective, I realize, but I don't think the gas itself is stimulating him to higher responses. This, of course, you could test, I guess. If some of my psychologist friends will help me out, that you could devise a test to determine if this is so, but this one sure doesn't--I wouldn't want to leave that impression at all.

DR BACK: Dr. Hodge presented a paper last year that preceded this where he presented the paper for Dr. Hine where this was done in humans. This was done in ten humans, and they did have impending unconsciousness at 16%. Two of his subjects did, and the EKG was abnormal, et cetera, but they showed depression and man shows simple depression. I don't think these monkeys do, not the thing you usually would see with an anesthetic type agent, and there's no analgesia involved, obviously.

MAJOR CARTER: Well, this again brings up the question of how do you determine if a human feels impending unconsciousness? Now, you can drink alcohol enough to the point where you feel you're getting impending unconsciousness. So this is really subjective, entirely dependent on the subject. It's possible you might be able to go to higher concentrations and not operate. I would say this is quite true, and not operate at all as far as any learned behavior, but really, it would be interesting to see if he would become unconscious as we think of unconsciousness associated with anesthesia. I don't know. This would suggest that he wouldn't.

MR. BIBIE: I wonder if you run corresponding performance or other tests on CBrF<sub>3</sub> using air as well as oxygen, would you have a variation in these tests by using smaller amounts of oxygen?

MAJOR CARTER: Well, true, you could. This is the reason we used the oxygen. We were not testing the effect of hypoxia on performance. We were trying to test the effect of the compound.

MR. BIBIE: On future spacecraft they may not use a hundred percent oxygen, they may use oxygen, mixed with nitrogen or other inert gas. I wonder if they used this Freon 113 what relationship you would have in performance with these environments?

MAJOR CARTER: I couldn't answer that, but remember that Dr. Hine's study was done in air.

CAPTAIN TAYLOR (Aerospace Medical Research Laboratory): As a chemist, I have to make one suggestion, not only about your paper but about other papers that are given more or less strictly from a medical viewpoint searching for cause-effect relationship, and this coincides more or less with what Dr. MacFarland offered on the CO



studies previously. It seems to me that it would enhance your study to a great degree if you certified the purity of the Freon 113 at the beginning, and listed the analytical method that was used - was it a well-worked out method, well-established? And then remove any doubt. In other words, if I wanted to be cantankerous I would say, "Well, are you measuring actually Freon 113, or are you measuring using a vapor fractometer a contaminant?" In other words, the unexpected low levels at which you're seeing your response would be enhanced if you backed it up by a very closely monitored analytical procedure. I have sat through so many medical papers where they blithely skip over the fact that--well, we analyzed and this should suffice. Error could be eliminated and the credence of the work would be enhanced, it seems to me, if just a few more sentences were added along this line.

MAJOR CARTER: I feel the other way. I'm sure your point is well taken, but when I listen to a talk I don't like to listen to a lot of routine facts. I assume that the investigator has satisfied himself of the purity and I assume that he has satisfied himself that the test is accurate. Otherwise, he shouldn't be there.

## CENTRAL NERVOUS SYSTEM EFFECTS OF BROMOTRIFLUOROMETHANE

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### INTRODUCTION

The purpose of this study was to investigate the central nervous system effects of bromotrifluoromethane ( $\text{CBrF}_3$ ) using electrophysiological methods and to compare these results with exposure to halothane, a close chemical congener and general anesthetic agent. Carter (1969) demonstrated performance decrements in monkeys trained on continuous and discrete avoidance performance tasks when exposed to 20-25%  $\text{CBrF}_3$  in oxygen. Van Stee and Back (1969) demonstrated that dogs exhibited excitement and tremors at concentrations as low as 20%  $\text{CBrF}_3$  in oxygen and convulsions at concentrations of 50%  $\text{CBrF}_3$  and higher. These convulsions were blocked by thiopental anesthesia. Hine et al (1968) further reported that at concentrations of 10%  $\text{CBrF}_3$  in air, human volunteers showed decrements in performance.

Those subjects who had previously been exposed to general anesthetics or had consumed moderate quantities of alcoholic beverages were familiar with the sensations produced. These subjects were described as being in the beginning stages of inebriation. It was postulated by the authors that loss of consciousness would occur at 20 to 25%  $\text{CBrF}_3$ .

This study was designed to evaluate the electrophysiological changes induced by  $\text{CBrF}_3$  in an attempt to better understand the performance and subjective changes reported.

This report contains two sections. The first deals with the electroencephalographic changes induced by  $\text{CBrF}_3$ . The second deals with changes induced in the evoked cortical responses.

## ELECTROENCEPHALOGRAPHY

### Methods

Six adult beagle dogs weighing 6 to 10 kg and six adult monkeys (*Macaca mulatta*) weighing 2.5 to 5 kg were used. Under thiamylal anesthesia (20 mg/kg), the animals were tracheotomized and ventilated mechanically.<sup>1</sup> A polyethylene catheter was placed in the femoral artery and blood pH was monitored and maintained within normal limits by adjustment of the mechanical ventilator.

Bipolar electroencephalograms were recorded on a six-channel direct writing oscillograph<sup>2</sup> from stainless steel hypodermic needles inserted in the scalp over the frontal, temporal, and occipital cortex (Caveness, 1962). The animals were immobilized by the intermittent intravenous infusion of tubocurarine. Following recovery from anesthesia a control record was obtained and the animals were exposed to 70-80% CBrF<sub>3</sub> in oxygen and 1% halothane in air for periods not exceeding one hour. Chemically pure bromotrifluoromethane<sup>3</sup> and oxygen were metered from pressurized cylinders through calibrated flowmeters into a polyethylene mixing bag and then administered to the animal through the respiratory pump. Halothane<sup>4</sup> was vaporized quantitatively<sup>5</sup> in air and administered through the pump. To test for EEG activation, the bell in a spring-wound alarm clock was used as an auditory stimulus and a 100 watt incandescent lamp was used as a photic stimulus.

### Results

The EEGs obtained during the exposure were of greater amplitude and synchronization and were dominated by 6-9 cycle per second waves. CBrF<sub>3</sub> did not effect EEG activation in response to photic and auditory stimulation. The results were qualitatively similar in dogs and monkeys although the voltage recorded from the dog's scalp was smaller due to the thicker skull and more prominent overlying musculature.

The results are best illustrated with the following figures. Figure 1 illustrates the induction of the CBrF<sub>3</sub> effect in a monkey. Increased amplitude and synchronization appeared during the second minute of the exposure and became maximal during the third minute. The preexposure EEG amplitude in the monkeys averaged 25-50  $\mu$ V and increased to a maximum of 150-175  $\mu$ V during the CBrF<sub>3</sub> exposure.

1. Respiration Pump, Model 607, Harvard Apparatus Co., Inc., Dover, Massachusetts.
2. Polygraph, Model 5D, Grass Instrument Co., Quincy, Massachusetts.
3. Freon 1701, E. I. duPont de Nemours and Co., New York, New York.
4. Halothane, U.S.P., Ayerst Laboratories, Inc., New York, New York.
5. Fluotec, Cyprane Ltd., England, dist. by Fraser Sweatman, Inc., Buffalo, New York.

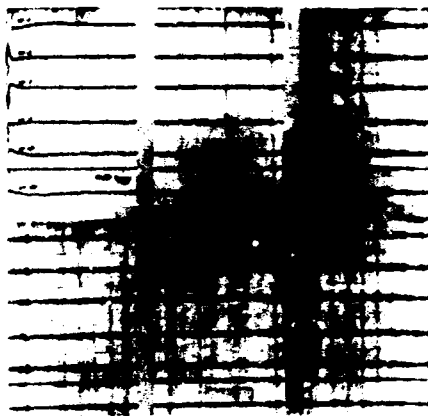


Figure 1. MONKEY E87. THIS FIGURE REPRESENTS THE INDUCTION OF THE CBrF<sub>3</sub> EFFECT. Beginning at the upper left are 0 minute (air), 1, 2, 3, 4, and 6 minute exposure to 70% CBrF<sub>3</sub>. Abbreviations used in the figures are frontal, F; temporal, T; occipital, O; right, R; and left, L. Positive is a downward deflection in all figures.

Figure 2 illustrates EEG activation by auditory stimulation. This animal was exposed to 70% CBrF<sub>3</sub> for 21 minutes and then stimulated. The presence of EEG activation, desynchronization, i.e., replacement of the slower, higher voltage waves by fast low voltage activity; is correlated with behavior arousal. Following EEG activation, the animal should be reactive to the environment.

Figure 3 illustrates EEG activation by photic stimulation. The animal was exposed to 80% CBrF<sub>3</sub> for 19 minutes and then stimulated. There was desynchronization of the EEG in response to turning the light both on and off.

Figure 4 contrasts the EEG effects of 70% CBrF<sub>3</sub>, 100% O<sub>2</sub>, and 1% halothane in the same monkey. Each exposure was for 60 minutes. Halothane produced a delta wave pattern characteristic of sleep or anesthesia (Martin et al 1959). During halothane exposure, auditory, photic or nociceptive stimuli failed to produce EEG activation.

The EEG pattern produced by CBrF<sub>3</sub> could be the result of cortical and/or thalamic depression since the reticular activating system is not significantly depressed.

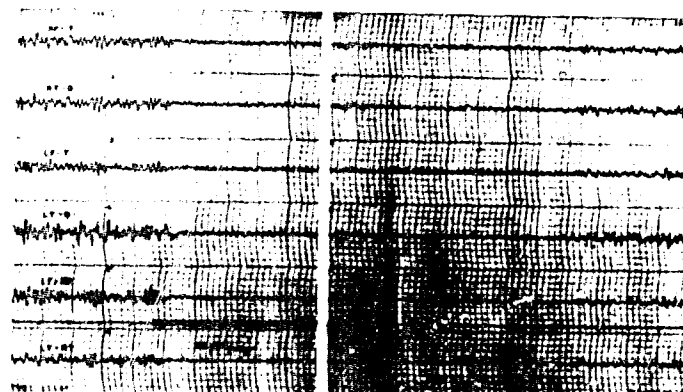


Figure 2. MONKEY E91 WAS EXPOSED TO 70% CBrF<sub>3</sub> IN OXYGEN FOR 21 MINUTES AND PRESENTED WITH AN AUDITORY STIMULUS. The heavy line indicates "stimulus on". Recordings are continuous except for a 5 second interval deleted to compress the illustration.

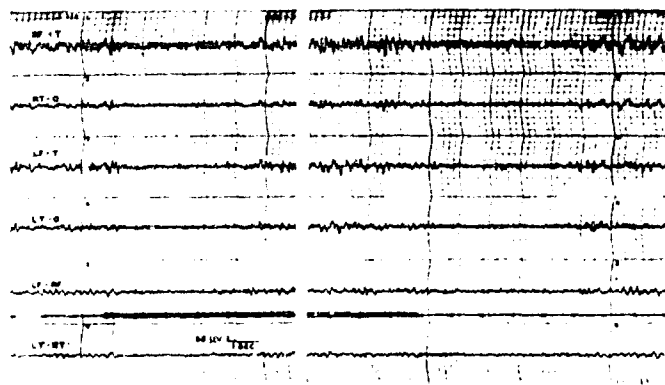


Figure 3. MONKEY E83 WAS EXPOSED TO 80% CBrF<sub>3</sub> IN OXYGEN FOR 19 MINUTES AND THEN CHALLENGED WITH A PHOTIC STIMULUS. The heavy line indicates "light on". A 9 second interval is deleted to compress the record.

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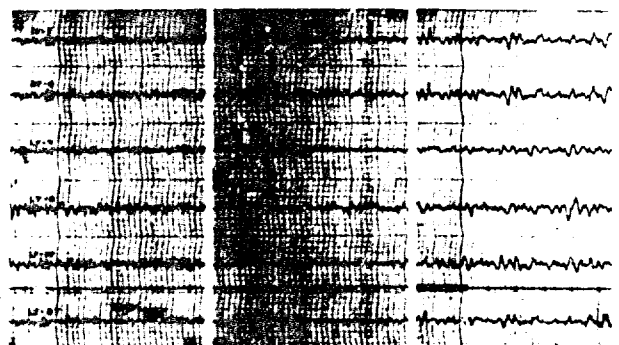


Figure 4. MONKEY E95. THIS ANIMAL WAS EXPOSED TO 70% CBrF<sub>3</sub> AND 1% HALOTHANE FOR 60 MINUTES. These recordings were made after a 20 minute exposure. CBrF<sub>3</sub> is on the left; 1% halothane on the right. A comparable air-breathing control is illustrated in the center.

## EVOKED CORTICAL RESPONSES

### Methods

To evaluate cortical depression, evoked cortical responses were studied in nine adult monkeys weighing 2.5 to 3.2 kg. Allobarbital<sup>a</sup> (diallylbarbituric acid, 100 mg/ml) was administered intravenously in a dose ranging from 0.45 to 0.9 ml/kg. Atropine (0.2 mg/kg) was given subcutaneously to control bronchial secretions. Tracheotomies were performed on all animals and they were fitted with a unidirectional valve that permitted inspiration from a reservoir bag and expiration into room air. The animals were permitted to breathe spontaneously.

Polyethylene catheters were placed in the femoral artery for measurement of arterial pressure using strain gauge transducers.<sup>7</sup> Standard Lead II EKG and tachographs were recorded from subcutaneous needle electrodes. The sciatic nerve was exposed and two silver wire electrodes were placed for stimulation. The monkey was immobilized in a stereotaxic instrument.<sup>8</sup> A portion of the skull and dura were removed over the frontal, parietal and occipital areas. A bipolar EEG was recorded from the cor-

6. Dial with Urethane, CIBA Pharmaceutical Co., Summit, New Jersey.
7. Strain Gauge Transducer, Model P23AA, Statham Laboratories, Inc., Hato Rey, Puerto Rico.
8. Stereotaxis Apparatus, Model "U", Baltimore Instrument Co., Inc., Baltimore, Maryland.

tical surface in the frontal area with 2 silver wire ball-tipped (0.5 mm diameter) electrodes approximately 2-3 mm apart.

A silver wire ball-tipped electrode was placed on the postcentral gyrus for recording the primary cortical response. Two stimulating and one recording silver wire ball-tipped electrodes were placed on the occipital cortex for evaluating the direct cortical response. The stimulating electrodes were 1-2 mm apart, and the recording electrode was 3-4 mm from the stimulating pair. Five successive sweeps of the oscilloscope were photographed with a 90 millisecond delay between onset of sweep and stimulus application.

Both cortical responses were monopolar recordings. The indifferent electrode was a stainless steel screw placed in the frontal sinus. Both cortical responses were photographed from the screen of a cathode ray oscilloscope<sup>9</sup> equipped with a low level differential amplifier. A constant-current stimulator<sup>10</sup> with a positive square wave was used to evoke the cortical responses. Arterial blood pressure, EKG, tachograph and EEG were recorded on a 4 channel direct writing oscillograph.<sup>2</sup>

Figure 5 illustrates the normal pathway and primary cortical response (under barbiturate anesthesia). The sensory nerves in the sciatic nerve are stimulated by a constant current square wave with a frequency of 0.25 cps, 0.5 milliamperes current, and 1 millisecond in duration. The nerve impulses are carried in the primary conduction pathways to the thalamus. This sensory information is then projected to the cerebral cortex and may involve 3-4 orders of neurons (Ganong, 1965).

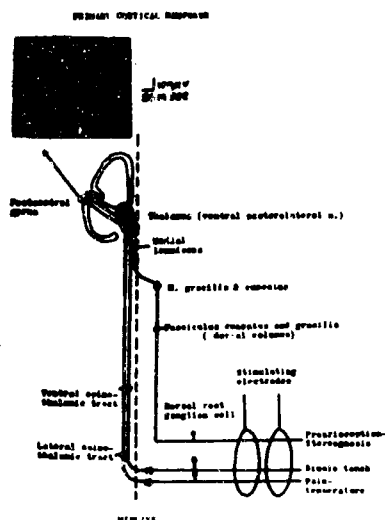
From the cortical surface, a positive-negative wave is recorded. The positive wave is thought to be the summation of the depolarizations of sensory terminals in the lower layers of the cortex. The negative wave is thought to represent depolarization of apical dendrites ascending from the depths to the surface of the cortex (Li et al, 1956). Cortical depression is represented by a decrease in one or both component waves, the negative wave being the more sensitive.

Figure 6 illustrates the mechanism of production and appearance of the direct cortical response under barbiturate anesthesia (Li et al, 1962). The cortical surface was stimulated with a positive square wave of 0.05 msec duration, 10 milliamperes strength, with a frequency of 0.25 cycles per second. This stimulation produced depolarization of axons in the molecular layer. This in turn produced dendritic potentials in the pyramidal cells which are recorded from the surface as a negative wave.

The origin of the second negative wave remains obscure. However, cortical depression is manifest by depression of one or both waves, the second wave being more sensitive.

9. Oscilloscope, Type 532, Tektronix, Inc., Portland, Oregon.

10. Constant-Current Stimulator, Model 7150, Nuclear-Chicago Corporation, Des Plaines, Illinois.



**Figure 5. MONKEY J-96 UNDER BARBITURATE ANESTHESIA BREATHING AIR.** The photographs of the evoked responses represent 5 successive sweeps of the oscilloscope beam with a 40 millisecond delay between onset of the sweep and application of the stimulus. Positive is a downward deflection in all figures. This drawing illustrates the pathway involved in generating the primary cortical response.

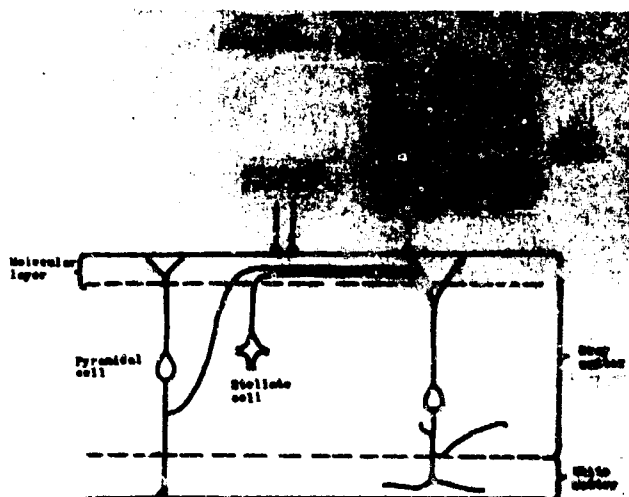


Figure 6. MONKEY K-30 UNDER BARBITURATE ANESTHESIA BREATHING AIR. The photograph represents the typical direct cortical response. The drawing illustrates the pathway involved.



After a 30-minute air breathing control period,  $\text{CBrF}_3$  was administered at concentrations of 20 to 80% in oxygen for periods of 10 to 30 minutes. One percent halothane in air was administered for 20 to 30 minute periods. Arterial blood gases were monitored before, during, and after the exposure. However, no attempt was made to correct acidosis or hypoxia if present.

### Results

Figure 7 illustrates the EEG and cardiovascular effects of a 30 minute exposure to 60%  $\text{CBrF}_3$  in oxygen and 1% halothane in air in the same animal under barbiturate anesthesia. Both agents have significant depressant effects on the cardiovascular system with bradycardia and hypotension. However, the EEG (under barbiturate anesthesia) is unchanged with  $\text{CBrF}_3$  while 1% halothane resulted in total suppression. The suppression of spontaneous EEG activity is a property of anesthetic agents in large doses (Martin et al, 1959). There was no hypoxia, acidosis or hypercarbia present during either exposure.

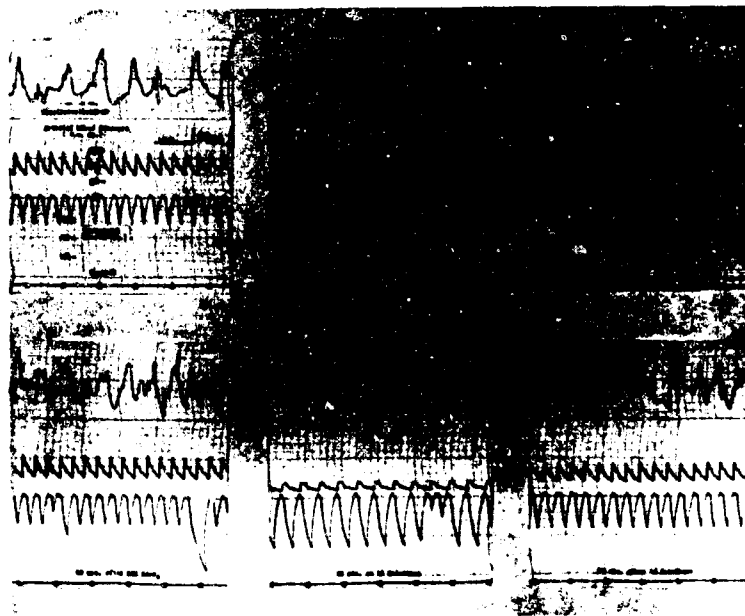


Figure 7. MONKEY H-29 UNDER BARBITURATE ANESTHESIA. The top three segments from left to right represent air breathing control, 30 minutes exposure to 60%  $\text{CBrF}_3$  in oxygen, and 60 minutes after the exposure ended. The bottom three segments from left to right represent the air breathing control 60 minutes after the  $\text{CBrF}_3$  exposure was concluded, 30 minutes exposure to 1% halothane in air, and 60 minutes after the exposure ended. The irregularities in the tachograph are artifactual. The electrocorticogram is a bipolar recording from the cortical surface of the frontal lobe.

Figure 8 illustrates depression of both the direct and primary cortical responses by 1% halothane in air. At 30 minutes, the responses are approaching the stimulus artifact baseline. These findings are consistent with cortical depression.



Figure 8. MONKEY H-29 UNDER BARBITURATE ANESTHESIA. This series of photographs illustrates the effect of a 30 minute exposure to 1% halothane in air on the evoked responses followed by a 30 minute recovery period.

Figure 9 illustrates the depression of both the direct and primary cortical responses by a 10 minute exposure to 60% CBrF<sub>3</sub>. The effects of CBrF<sub>3</sub> are less striking than those observed with halothane. There is flattening of the second wave of the direct cortical response and a decrease in all components of the primary cortical responses.

Similar results were seen in all animals. When the initial depth of anesthesia was sufficient to cause significant respiratory depression, (respiratory acidosis) and hypotension, 60 to 80% CBrF<sub>3</sub> produced profound hypotension and hypoxia, and the cortical responses and EEG resembled halothane. This probably represented the effects of hypoxia and hypotension rather than CBrF<sub>3</sub>.

When hypoxia and extreme hypotension were not produced, 20, 40, and 60% CBrF<sub>3</sub> produced increasing levels of depression of the primary and direct cortical responses. The maximum effect can be seen in figure 9.

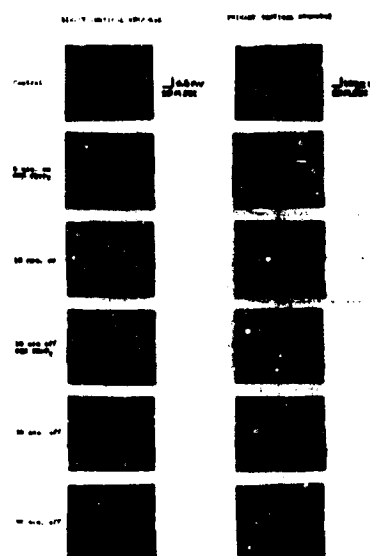


Figure 9. MONKEY H-41 UNDER BARBITURATE ANESTHESIA. This sequence of photographs illustrates the effect of a 10 minute exposure to 60%  $\text{CBrF}_3$  in oxygen on the evoked responses followed by a 30 minute recovery period.

A 10 minute exposure was sufficient to produce the effect, but 20-30 minute exposures resulted in greater decreases in the evoked responses.

### CONCLUSIONS

The data presented support the hypothesis that  $\text{CBrF}_3$  produces cortical depression with relative sparing of the reticular activating system, permitting alterations in performance without loss of consciousness. It is possible that a component of thalamic depression is also present contributing to EEG slowing and decreased amplitude of the primary cortical response. Further experiments are necessary to evaluate this hypothesis.

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## DISCUSSION

DR. COULSTON: What would happen, just for instance, if you did these similar experiments with indwelling catheters, etc., on intact, unanesthetized animals? Do you think you'd get the same data?

CAPTAIN CHIKOS (Aerospace Medical Research Laboratory): Unfortunately, you can't do the direct and primary cortical responses in unanesthetized animals because it requires cortical depression and idling population of cells to generate them, so that what you have to do, if you're going to investigate this sort of thing, would be to have chronic indwelling extracellular or intracellular electrodes and measure the activity from the individual cell. At this point in time it should be done. However, it requires expensive equipment and quite a degree of sophistication by the investigators. We are contemplating this at this point. We may let some other national facility do it.

DR. COULSTON: It should be done.

CAPTAIN CHIKOS: It should be done. I agree, but I don't think we have the capability at this time to do it.

DR. HODGE: Is that the same question as what is the barbiturate effect on the reticular activating system in your experiment? Is that the same as Dr. Coulston's question?

CAPTAIN CHIKOS: I'm not sure. I might say that with barbiturates, the reticular activating system is indeed depressed. However, when it is done in air or oxygen, the reticular activating system seems to be very functional up to 80% CBrF<sub>3</sub>--the point being that either halothane or barbiturate anesthesia knock out the reticular activating system and allows one to study the cortical activity, per se, without these interfering influences, and we established this protocol in an attempt to study the cortical activity.

MR. WANDS: I think it's important to recall from last year, Dr. Hine's work, presented by Dr. Hodge, that humans exposed did not show truly aberrant behavior in the range between 10 and 15% CBrF<sub>3</sub> in air. They had sensations of slight inebriation, ultimately impending sense of blackout, but during the entire period of exposure, they were able to perform assigned tasks, could take direction, leadership, et cetera, that was given to them, without any evidence of any aberrant behavior.

CAPTAIN CHIKOS: Right. I stand corrected. That's what the paper stated. I was thinking in terms of Dr. Carter's animals that were getting up and crawling around all over the cage. That was directed more in that line than towards the people.

MR. WANDS: Perhaps humans are more inclined to behave themselves even when drunk.

DR. HODGE: I noticed in the post recovery record of the  $\text{CBrF}_3$  heart, that there seemed to be a variation in rhythm.

CAPTAIN CHIKOS: Unfortunately, that was a variation in the sensitivity of the tachograph. The monkey EKG is very small, voltage-wise, and the sensitivity knob on the tachograph sometimes goes out of order and we sometimes get a fluctuation in the tachograph record. However, look at the blood pressure, nice and regular, saying no change in rate.

DR. THOMAS (Aerospace Medical Research Laboratory): I just want to recall from last year's conference report that we have seen spontaneous arrhythmias, A-V blocks, spontaneous fibrillation, with this compound in dogs. Now, the old question, of course, in using a compound like this is what risks you want to take? Do you want to get fried? Are you going to take a chance of passing out for a short time? or, if you have time to grab an oxygen mask, you won't have any troubles. It's the best compound we have for putting out fires in oxygen atmospheres, so we are back to the old point of trade-offs. The recovery is so fast that, really, if you can put out that fire before it really gets started and you can go on oxygen quickly, after that you can dump the atmosphere and repressurize. It's between making or breaking the mission. So, it's not a nice compound but it does the job.

DR. HODGE: I suppose the solubility of this compound in blood is extremely low, is that true?

MR. WANDS: It seems to wash out very rapidly.

DR. BACK: Dr. Kaplan is studying that area.

LT. COLONEL KAPLAN (Aerospace Medical Research Laboratory): It is very insoluble in water and blood and we did some tissue studies of brain and heart  $\text{CBrF}_3$  content after exposure. During the first minute postexposure, approximately 95% of the  $\text{CBrF}_3$  was eliminated from the blood, brain, and heart tissue--a very marked falloff of  $\text{CBrF}_3$ .

DR. BACK: That's another paper next year.

MR. WANDS: Ninety-five percent of how much? What's the actual value?

LT. COLONEL KAPLAN: I believe it was in the neighborhood of 300 micrograms per gram of tissue.

MR. WANDS: From what exposure concentrations?

LT. COLONEL KAPLAN: Eighty percent  $\text{CBrF}_3$ , anywhere from 5 to 30 minutes.

DR. HODGE: Thank you very much, Captain Chikos. Now, we have a brief period for general discussion on any of the papers or any discussions that anyone wants to bring up at this time. Is there someone who didn't get his question in a little while ago who'd like to raise it now? I would like to ask for one point of explanation. Can someone tell me the difference between the time interval estimation in this carbon monoxide study and the method used by Beard?

DR. MIKULKA: It was essentially identical. The procedure lasted for three minutes and the subject didn't know how long it was working. He was told to go and estimate ten second periods without external or audio cues. In our study he pressed the lever at what he thought were 10 second intervals and that was electronically recorded. After the first estimate outside of three minutes, he was told to stop. He never knew how to gauge estimations. We patterned this almost identically after Beard's work.

DR. HODGE: I'm sorry, I'm not quite with you. What was the stimulus? Where did the time estimation start? Did the man listen to a beep or clock tick?

DR. MIKULKA: No, it was voice command to the subject to start time estimation trial. In each testing interval the subject had five tracking tasks, about a minute per shot on a control stick, and then went to time estimation back to tracking, and it took 15 minutes for the whole block of time. After tracking was finished, he rested a few seconds and then I said, "All right, on my signal, you must start time estimation", and then I'd give them GO. He then started, however he did it, to estimate ten seconds, and then tapped a switch with the right hand and started to estimate ten seconds again. So, after my initial signal, it was self-repeating on his own signal. After he did the first one past the three minutes we said, "Okay, stop". The data was the mean-time averaged for all those intervals. I hope that clarifies it.

DR. HODGE: Thank you.

LT. COLONEL STEINBERG: In dumping this Freon into the cabin atmosphere-- this has nothing to do with the pharmacology or physiology involved, but the mechanics, -- is there any system that's been fixed on yet? Is it a light sensing system? Have you found any one system to be better than any others, or haven't you decided on a system yet?

DR. BACK: There are pros and cons as to how--whether you want to dump it automatically or not, so that some people think that the pilot ought to be the actuating device in an aircraft or in a close environment, and the other way is to do it by UV sensors and that will dump it in a matter of 20 milliseconds. Now, you've got all kinds of problems, depending on use. If you're talking about a 747, which the FAA was interested in equipping with  $\text{CBrF}_3$ , you can imagine that if they dump the whole contents to reach a 2% concentration in the entire cabin and it's done from the periphery

of the 747 cabin, obviously, the guy over there on the side would be getting maybe a hundred percent in his face, while the guy out here on the aisle seats wouldn't be getting a fraction of that for some milliseconds. So you would have a total gradation of a hundred percent on the sides to 7% in the middle of the room.

MR. WANDS: Extending over what period of time?

DR. BACK: Well, extending over the period of time that it takes to deliver it and the time it mixes with the cabin air. If you're in a crash situation, how long does it take to break apart? I don't know. Of course, this could have been lifesaving, for instance, in the 727 crash at Utah, because these people weren't all killed by the impact. Most of the people died from fire and smoke after the impact. Some of them were out of their seats, as a matter of fact, and could not get out. The matter of how you do it is depending on what the situation is. Some people think, especially in space cabins or in aircraft, that the pilot ought to activate the system because you're worried about false alarms from the sensors. We had this problem with water. Some of my sergeants will attest to that. They were deluged with 350 gallons of water in 20 seconds. In fact, Colonel Kaplan and Captain Chikos here, they're pioneers in this. They actuated the system in the dome inadvertently in one instance by a UV light source which they thought was a flashlight, but, it was a device for testing the UV sensors built in a flashlight case.

LT. COLONEL KAPLAN: There are some other systems too.

DR. BACK: There are other systems, smoke sensors. This is an area which the people in Ground Safety, Ground Support, are working on at Wright-Pat and other places. I can't give you all the details, but they're looking at all the sensors - smoke, flame, spark, even temperature.

DR. THOMAS: There's only one problem with automatic triggering. In aircraft, the no smoking signs must be on all the time. In aircraft, you know, you don't have the problem of oxygen rich environment and I think there's plenty of time for the pilot to punch a button. In spacecraft it's a decision we'll have to face.

DR. BACK: The pilot is a far distance from where the fire may be in the 747. All I'm saying, the alarm is an easy way out in the engine nacelle or someplace like that, but it sure is a devil of a tough problem when you get back with that cast of hundreds of passengers.

DR. THOMAS: Maybe temperature sensors wouldn't be so bad, really, placed across the cabin to sense hot spots.

DR. HODGE: Mr. Wands says it's maybe a panacea in a crash situation.

CAPTAIN SCHWARTZ: Dr. Back, you're going to be using behavioral systems for testing environments with monkeys in the future, I'm sure. It seems that you're under fire from the psychologists saying that under threat of death in the last breath, they're going to pull this lever. I am not sure that it is because they get this response



or they know the 15 minutes is coming so they start working, shock or no shock. Is there any way to calibrate the system so that you know significant responses and you know that your compounds are doing something to them?

DR. BACK: Well, it's pretty obvious that we're using a very robust situation here, as Major Carter indicated. It's obvious, though, the CO animals are still responding where the CBrF<sub>3</sub> animals were not responding to the same sort of task stimulus, so it is very robust, agreed, but it's the only thing we had at the moment. Now, we recognize full well that we need something very much more sensitive and we are working on that right now--Dr. Carter went down to Arizona State University to test a new system. We think we have a much more meaningful task now, a more sensitive task, in which the animals are given a time out and time in period, and they are still negatively reinforced. In other words, we are still using shocks because I'm always worried toxicologically about loss of appetite in a positive reinforcement situation. After all, if you take an animal's appetite away, he's not going to perform just to get food, so you're always going to get a depression of effort from a compound which does work against appetite. So we still are keeping with the negative reinforcement. But it looks like this test with the time in and time out interval, where the animal is given a start cue, and he's got, say, from the third to the fourth minute with time out. On the fourth minute he starts working, and he must keep working at an increasingly rapid rate until the last 3/10 of a second or he is given a shock if he isn't pressing at that last 3/10 second. This has been tested out with decaborane, as a matter of fact, and it looks like a number of things can be gleaned from this. Number one, the animal may be so confused that he works in the time out area, from the third to the fourth minute. He's pressing levers and he doesn't have to. And then the other thing is the latency with which he starts to work after the fourth minute, and then the rapidity with which he goes towards his final goal which is the last 3/10 of a second. Now, that last 3/10 of a second can be changed as the compound dictates, and I think this is going to be a much more meaningful way of doing it. We're still going to have some discrete avoidance tasks piled in on top of this, but this looks very, very sensitive, and it has a time estimation in it which everybody looks at as being one of the important criterion for toxicological study.

MAJOR CARTER: There are disadvantages to the type of task we're using, and the one Dr. Back is talking about has an advantage. When I am exposing the animal, -- let's say he misses the auditory cue, he gets a shock. He doesn't know exactly why he gets the shock so he just starts beating heck out of the lever. You know, he just presses that lever and he misses the next one, and he presses it that much harder. So, really, over the entire 15 minute period, when you superimpose results one on top of the other as we did here, it confuses the issue. I think if I did it over again, I'd only have maybe auditory and visual, present it once every 30 seconds, and so forth; whereas, the new task Dr. Back talked about, the animal only gets one shock regardless, so you can determine performance over this entire period without the animal getting reinforced right in the middle and confusing the whole issue.

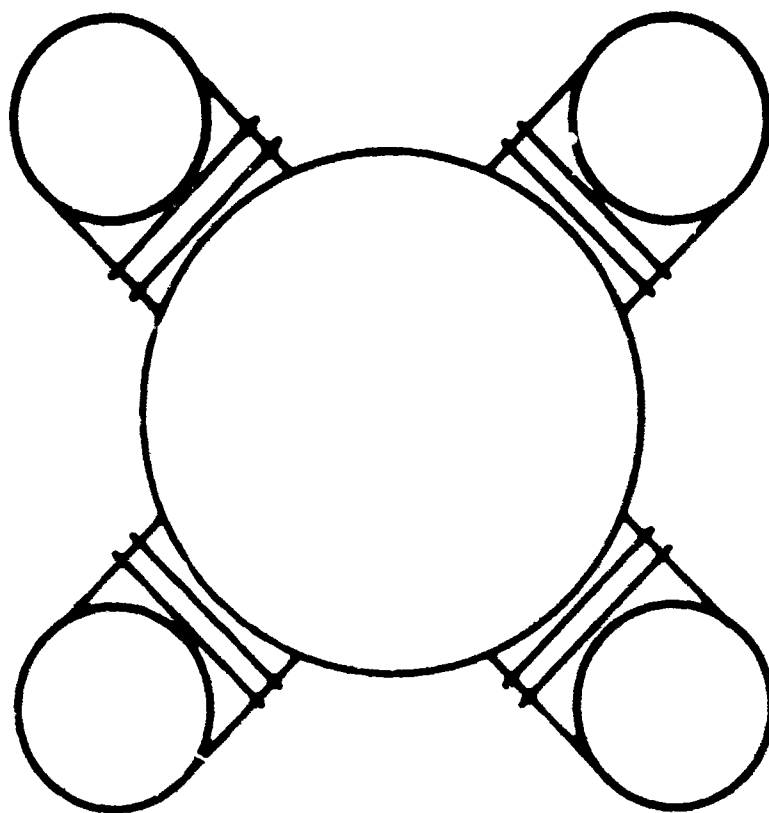
DESIGN AND CONSTRUCTION OF A SECOND GENERATION  
ALTITUDE EXPOSURE FACILITY

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Since this presentation consisted primarily of orienting the group on the new altitude facility prior to the tour, no formal paper was prepared by the author.

The essence of the presentation was the explanation of the functions which can be accomplished by the interconnecting airlock pictured diagrammatically below.



INTERCONNECTING AIRLOCK - THOMAS DOMES, LOWER LEVEL.

The main purpose for the large airlock, approximately the same size as a dome, is to enable investigators to carry out physiological measurements, surgical preparations for pharmacological procedures, biopsy and necropsy procedures, and pulmonary and cardiovascular function measurements on animals exposed in any of the four domes without change in such important environmental parameters as altitude, gaseous composition of the artificial atmosphere, contaminant concentration, etc.

It also allows sequential cross exposures to various contaminants because animals can be moved from one dome to another without ever being repressurized to ambient atmosphere.

Another potential use is for short term human volunteer exposures which do not interfere with dome operations since the individual airlocks can still be used independently for access to each dome.

This new capability will play an essential role in eliminating the potential for artifact formation in the morphological and ultra structural investigation of lung changes observed in long term studies with pure oxygen and mixed gas atmospheres because the necropsy and lung fixation for morphometric studies now can be accomplished in the actual atmosphere and at the same total pressure to which the animals were exposed.

The presentation was followed by a tour of the new altitude and laboratory facilities of the Toxic Hazards Division.

## INHALATION TOXICITY OF ETHYLENE GLYCOL

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### INTRODUCTION

This paper is an informal progress report covering a two year study of the toxicology of ethylene glycol. In August 1967 it became evident there would be a number of places within the Apollo spacecraft where an ethylene glycol leak could occur. Because of its low vapor pressure and maximum 8 hour continuous exposure, ethylene glycol was not considered an industrial toxic hazard by the inhalation route. However, there was a dearth of information on continuous inhalation exposures of the type which might occur in the spacecraft. Further, satisfactory methods for the determination of airborne ethylene glycol were nonexistent. A two-fold problem existed.

1. To gain information on the long term toxicity of ethylene glycol with respect to humans.
2. To find an adequate method of detection and determination of glycol.

Neither gas chromatograph nor colorimetric methods were suitable for the confines of a spacecraft.

### Toxicology of Ethylene Glycol

Before humans could be exposed to ethylene glycol vapors, it was essential to obtain background information on inhalation toxicology of ethylene glycol. Ethylene glycol ingestion initially results in CNS depression, and acutely, death results from respiratory paralysis. The cause of death from chronic ethylene glycol was renal failure, apparently the result of oxalate deposition in the kidney.

Our initial studies consisted of the exposure of rats, rabbits, mice, beagle dogs, guinea pigs, and monkeys in an oxygen atmosphere at 5 psia saturated with ethylene glycol. Twenty-one days of exposure produced no discernable responses. Concurrently, a group of trained Rhesus monkeys was exposed to the same atmosphere. Over a period of three weeks no changes in their avoidance response behavior were observed.

In an orderly progressive study, it was essential to examine both sensitive behavioral parameters and the metabolism of glycol in a variety of species before going to human exposures. In a collaborative effort by the 6571st AMRL at Holloman AFB, Baylor University, and the Institute for Experimental Toxicology and Pathology at Albany Medical College, chimpanzees, baboons, and a variety of lower animals were studied both from the metabolic and the behavioral aspects. A psychological test system for chimpanzees was designed at Holloman AFB, the results of which are reported in a separate paper by Captain Felts. However, there were no behavioral changes noted.

Chimpanzees were injected with ethylene glycol and the metabolism of ethylene glycol followed. Ethylene glycol may be oxidized to glycolaldehyde, thence to glycolic acid, glyoxylate, and oxalate. This is the pathway that leads to oxalate crystal deposition. The glyoxylic acid may also go to glycine by transamination and ultimately to carbon dioxide.

Ethylene glycol was given to a number of animals per os and by intravenous injection. The baboon excreted the major portion of the administered glycol unchanged. A small amount of oxalate was found. Rats given 1 ml/kg of  $^{14}\text{C}$  ethylene glycol per os excreted virtually no oxalate. The major portion of the material was excreted unchanged as glycol. The Rhesus monkey excreted approximately 80 percent of ethylene glycol unchanged in 24 hours. However, 18 to 20 percent of the material was excreted as oxalate and a trace of glycolaldehyde was detectable. Approximately 60 to 75 percent of the isotope was accounted for.

A major portion of the material found in the urine after intravenous administration to the chimpanzee appeared in the urine as oxalate. A small quantity appeared as unchanged ethylene glycol. Approximately 75 percent of the radioactivity in the urine of the chimpanzee was in the oxalate. Other animals excreted 70 to 75 percent as unchanged glycol. It was apparent, therefore, that the chimpanzee metabolized ethylene glycol differently from the Rhesus, rat, or baboon.

Tissue distribution of ethylene glycol was studied in the monkey. The cerebrum, cerebellum, heart, kidneys, lungs, liver, and spleen levels were close to serum levels and indicated a distribution in the tissues by a rapid and even distribution of ethylene glycol through the body water. Pancreas and ileum levels were higher than other organs. There is no explanation for the high pancreas level, but the high ileum level was most probably due to slow absorption.

A group of monkeys were exposed to an aerosol exposure of 500 mg per cubic meter for a period up to 30 weeks. Of these animals, six died of typical chronic ethylene glycol symptoms. Two survivors were retained for a period of 30 weeks. Neither exhibited overt symptomatology for two days. Those that died exhibited impacted intestines, oxalate crystals in the kidneys, and a deposition of oxalate crystals in the lungs. As observed previously, ethylene glycol was fairly evenly distributed in the tissues and organs--the level in body water running about 1 to 1.25 that of the serum.

During the course of the exposure an increase in blood creatinine was observed. BUN increased as much as 10 fold before the animal became moribund.

With the background of experience that was obtained, it was thought that we could proceed cautiously to human exposures. A group of male volunteers 20 to 30 years of age was exposed to aerosolized ethylene glycol for periods up to 28 days. Blood chemistries were followed and the excretion of urea and creatinine carefully observed. Simultaneously, a group of psychomotor and a battery of psychological tests were performed. Psychiatric evaluations were made to detect personality changes. As a result of these human exposures an organoleptic technique was developed for the detection of ethylene glycol. Volunteers exposed to 25 ppm were completely oblivious to the presence of ethylene glycol. Blood levels of ethylene glycol were not significantly above preexposure blank levels. The psychological, psychomotor, and psychiatric tests showed no changes. As part of the protocol, levels were run up to 50 ppm for short periods of time. Although ethylene glycol has no odor, it does have a very distinct taste. At 50 ppm, the subjects, unaware that the level had been raised, began to taste its sweetness and experienced an irritation in the pharyngeal area. When the ethylene glycol was raised, without their knowledge, to 75 ppm, several were awakened out of their sleep. They could not tolerate the exposure.

Although these subjects could not be exposed to higher levels of ethylene glycol continuously because of the taste and irritation, no observable changes took place when the level was below that of organoleptic detection. Thus a built-in detection system was found which obviated the need for further toxicological evaluations in men. The problem became an operational one rather than a toxicological one.

Each astronaut is exposed to ethylene glycol fumes and aerosol prior to launch. He learns the distinctive taste and pharyngeal response. If in flight the crew cannot detect glycol, no problem exists. If they do detect glycol they are to don their space-suits and isolate themselves from the cabin atmosphere. The mission may then continue with no concern over ethylene glycol toxicity.

## DISCUSSION

MR. VERNOT (SysteMed Corporation): Even though ethylene glycol has a fairly low vapor pressure, saturation vapor pressure at room temperature gives concentrations of over 200 parts per million, and unless this was a very short acting experiment-- What I mean is that the people or the animals breathed the vapor rather quickly after the aerosol was formed, --it's hard for me to see that the aerosol could exist for any time. It should vaporize if you have 50 to 100 parts per million concentration.

DR. HARRIS (NASA, Houston): Although the vapor pressure is low, as a matter of fact this is one reason why you're going to maintain an aerosol. The level is unpredictable in terms of what we're going to get out of the cooling system in the spacecraft and so what we're looking for is a level which is detectable. This is what we're concerned with. Now, we've got, I believe, something like 9 pounds of ethylene glycol in that system and with a pinpoint leak, you can maintain an ethylene glycol atmosphere in a spacecraft for a long period of time.

MR. VERNOT: What I don't understand is the fact that you could get a stable aerosol at 50 to 100 parts per million of ethylene glycol. It should go into the vapor form.

DR. HARRIS: Immediately after it's released by pinpoint leaks it is an aerosol, yes. Then it goes into the vapor phase. I'm not sure I quite get what you're driving at.

MR. VERNOT: Well, it's just that if you measure your concentration of 50 to 100 parts per million, you should have a vapor, not an aerosol, unless--

DR. HARRIS: You've got continuous introduction.

FROM THE FLOOR: How many human subjects were used? Could you give us a brief description? Were they male or female?

DR. HARRIS: I think the best man to answer the question is Dr. Coulston.

DR. COULSTON: Actually, to answer the question that's been raised just previously, the aerosol is put into the exposure room through five jets under a continuous air pressure varied between 40 and 60 pounds per square inch so that we had a considerable pressure, and believe me, when you get above 50 parts per million, the room is nothing but a fog. We measured this continuously at five stations within the

room throughout the experiment at least three or four times a day, so we had a pretty good idea of the distribution within the room. In the total experiment we did two phases. One phase was a pilot run to make sure all the systems were go, as you people say, and furthermore, to protect ourselves against any unforeseen hazard, so a few men, four men, plus a control group, were run for a period of approximately a week, and then the total group of approximately 34 men were put on the test. The controls were in another part of the building, and the actual experimental group was 28, I believe. Now, these men were followed continuously throughout the exposure. I think Dr. Harris has given an adequate account, very complete account of the experiments as they were done.

DR. HODGE: This is 24 hour a day exposure?

DR. COULSTON: The men actually were on the test approximately 22 hours a day. They were allowed to go out of the room particularly on Sundays when they may have had some visitors, but sometimes it was as high as 23 hours a day in the room. You can't do it continuously; you got to give the men a chance to do something else. So they took their meals outside the room, you see, and that's what accounts for the time.

DR. THOMAS: My question is, this fog you noticed, this is a visible fog at 50 ppm. What was the relative humidity in the room, if--Suppose you put ethylene glycol in water to vaporize it?

DR. COULSTON: We studied the humidity but not in any great detail. We had a bar humidigraph in the room as well as a temperature bar graph. When the fog was visible throughout the room, I should say the aerosol was in the range of 0.5 to about 2 to 3 microns, 6 feet from the nozzles, I'll put it that way. This was determined by microscopic examination. We're not very sophisticated with this, so we had a calibrated microscope and did direct counts of the droplets as they appeared on the glass slide. We also prepared permanent record slides using a system developed, I believe, at Holloman, where the methylene blue was put onto the slide and the droplet was allowed to dry on the slide so that you got some idea of the size of the droplet. But the humidity, when you got above 50 parts per million, was roughly about 60 to 70 relative humidity. It was very high. But when you approached 70 ppm you couldn't see across the room. It was aerosol, and solid, and it stayed. It stayed for quite a long period of time.

DR. PIERSON: I was interested in the subjects, primarily. Were they astronaut candidates? Were they male college students, professionals? What age group? Who were they?

DR. COULSTON: These were prison volunteers in a prison. They consisted of both white and black of an age group approximately from 18 to 50. They were thoroughly examined to make sure they were healthy subjects as far as we could tell.



DR. LONGLEY (Institute of Gas Technology): At levels where you could notice a taste or see liquid droplets in the space cabin, would you not have to abort or fix immediately, regardless of the toxicological problem?

DR. HARRIS: There are times when you can't abort. If you're in translunar coast you can't; you go on through, around and back.

DR. LONGLEY: My point is the toxicology hazards to the human from the vapor exposure would probably be secondary in this case, would it not?

DR. HARRIS: No, because the engineering problem may not be a serious one. You want to check. For one thing, you're concerned if this is in your heat exchanger. If your heat isn't building up, then your temperatures aren't building up and there may be no cause for abort. This is going to have to be the judgment of the engineer at the console, as far as that part is concerned. That system may still perform and he may go on.

DR. LONGLEY: You also have a redundant heat exchanger?

DR. HARRIS: Yes. The one problem with this heat exchanger, with this glycol in the attenuator, is that there's no way really of telling how much there is. You can't tell what the extent of the leak is, in other words, to use that as a detector.

MR. WANDS: I would certainly like to congratulate you, Elliott, for riding herd on this program for NASA, also NASA for sponsoring this work. As you well know, the older literature on ethylene glycol was pretty horrible. We had some very bad information, some very scanty information, and there was no real quantitative measure of the extent and nature even of the toxicology of the material other than the ethylene glycol studies that FDA had done in the years gone by with the sulfa drug episode. Certainly the sort of work that you people have done is essential for proper environmental control planning. I would like to emphasize to this entire audience, especially those of you who have responsibility for introducing these materials into the system, that it is impossible even for the best qualified brains in the country to make reasonable guesses in the absence of any data.

LT. COLONEL KAPLAN: Dr. Harris, if I may ask a question, on the four-hour distribution study, was this a distribution of the ethylene glycol or total carbon 14?

DR. HARRIS: This is distribution of ethylene glycol.

## EFFECTS OF EXPOSURE TO ETHYLENE GLYCOL ON CHIMPANZEES

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### INTRODUCTION

Utilization of ethylene glycol as a heat exchanger in the Apollo system raised possible toxicological problems. Numerous reports (Friedman, 1962; Hageman, 1948; Mills, 1946; Nadeau, 1954; Pons, 1946) have indicated ethylene glycol is quite toxic to humans. The lethal dose for humans, by the oral route, has been estimated to be 1.6 ml ethylene glycol/kg of body weight. Information regarding effects of various routes of administration is limited primarily to that of accidental ingestion. Available information on species other than man is also limited. An inbred strain of mice (SWR/J) developed failure of a blood clotting factor following low level feeding of ethylene glycol (Allen, 1962). Rhesus monkeys were unaffected by chronic administration of ethylene glycol in the diet (Blood, 1962). Back (1967), Esparza (1967) and Vernot (1967) indicated inhalation of glycol is not harmful to the rhesus monkey.

This report discusses (1) the selection of the chimpanzee over the rhesus monkey for ethylene glycol toxicity studies and (2) the inhalation studies with the chimpanzee to determine the seriousness of a possible glycol leak in the Apollo system.

### METHODS

(1) Two chimpanzees were exposed to an ethylene glycol saturated atmosphere (256 mg/cubic meter at 70 C) for 28 days in a performance evaluation chamber. The saturated atmosphere was produced by an aerosol mist of less than 5 micron particles. The ethylene glycol was gravity fed to the chamber and forced through a nozzle in the ceiling with compressed air. The chamber altitude was 4300 feet, the ground level altitude of Holloman Air Force Base. (2) Four other chimpanzees were exposed to an atmosphere saturated with ethylene glycol vapor. These chimpanzees were in a Thomas Dome with a simulated spacecabin atmosphere of 5 psi, 68% oxygen and 32% nitrogen for 28 days.

Ethylene glycol levels in the performance chamber were measured by gas chromatography. A Porapak QS (80-100 mesh) 6 foot glass column was used in a gas chromatograph\*. Injector temperature was 240 C; column temperature 180 C; and hydrogen flame detector 270 C. Flow rates were: helium carrier gas 50 cc/min; hydrogen 45 cc/min; and air 450 cc/min. The chamber was sampled by drawing air from the chamber into a 150 ml gas collecting flask. Air samples from the flask were gently bubbled through water with dry nitrogen. The method proved satisfactory for full recovery of ethylene glycol into a volume of water as small as 0.25 ml. This aqueous solution was injected into the chromatograph. A similar procedure was used in monitoring the Thomas Dome (Vernot, 1967).

Hematologic and serum chemistry studies were done on the performance test animals before they were placed in the chamber and again upon their removal. Urine concentrating ability was also measured at these times in the following manner: food and water were withheld for 36 hours after which time (1) a urine sample was collected by catheterization and (2) a blood sample was collected. Serum and urine osmolality were measured with a freezing point depression osmometer\*\*.

Hematologic and serum chemistry values were determined on the chimpanzees in the Thomas Dome at weekly intervals. Renal function tests, inulin and para-amino-hippuric acid (PAH) clearances, and the PAH tubular maximum excretion rate, were done according to Smith (1954). Each test was done four times on each chimpanzee: (1) at Holloman Air Force Base prior to their transport to Wright-Patterson Air Force Base, (2) at Wright-Patterson Air Force Base before entering the dome, (3) at the midpoint of exposure in the dome, and (4) immediately following the 28 day exposure.

Heyrovsky's (1956) indole-3-acetic acid method was used to determine inulin concentration in urine and protein free filtrates resulting from 10% trichloroacetic acid precipitation of serum. PAH concentration in urine and cadmium sulfate protein free filtrates of serum was determined according to Chasis (1945). The test for urine concentrating ability was not done on the chimpanzees in the Thomas Domes because of logistics requirements.

## RESULTS AND DISCUSSION

Effects of intravenous injection of ethylene glycol in rhesus monkeys and chimpanzees as described in tables IA and IB indicate that the rhesus monkey is much more tolerant of the glycol than is the chimpanzee. The dose of 2 ml ethylene glycol/kg body weight, lethal to the chimpanzee, caused only mild ataxia in the rhesus monkey. Of these two non-human primates, the chimpanzee reacted to the ethylene glycol in a manner more comparable to the reaction of man, based on the levels of oral toxicity reported for man. Therefore, the chimpanzee was chosen as the test animal for further experimentation.

\* Barber Coleman Model 5000

\*\*Advanced Instruments Model 65-31

TABLE I A

I. V. INJECTION OF ETHYLENE GLYCOL  
(Rhesus Monkey)

DOSE	EFFECTS
2 ml/kg	Ataxia for 2 hours Vomiting with recovery after 2 hours Oxalate crystals in kidneys after 2 wks
1 ml/kg	No behavioral changes No clinical signs of illness Oxalate crystals in kidney after 2 wks

TABLE I B

I. V. INJECTION OF ETHYLENE GLYCOL  
(CHIMPANZEE)

DOSE	EFFECTS
6 ml/kg	Ataxia; coma Death in 10 1/2 hours Oxalate crystals in kidneys at autopsy
2 ml/kg	Ataxia; coma Death in 12 3/4 hours Oxalate crystals in kidneys at autopsy
2 ml/kg	Ataxia; coma Death in 9 3/4 hours Oxalate crystals in kidneys at autopsy
1 ml/kg	Ataxia; pulmonary complications Death in 37 1/2 hours Oxalate crystals in kidneys at autopsy
1 ml/kg	Ataxia; vomiting; recovery Sacrificed at 48 1/2 hours Oxalate crystals in kidneys at autopsy

Two chimpanzees were trained to perform two tasks inside the psychopharmacological test chamber (figure 1). The first task was an auditory discrimination task in which the animal was required to locate the source of a sound and press the appropriate speaker to shut off the sound. A food pellet reward was given after each response. Speakers were located on three sides of the chamber; each speaker emitted a different sound frequency. Both the number of correct responses and the time required to make the response were measured.

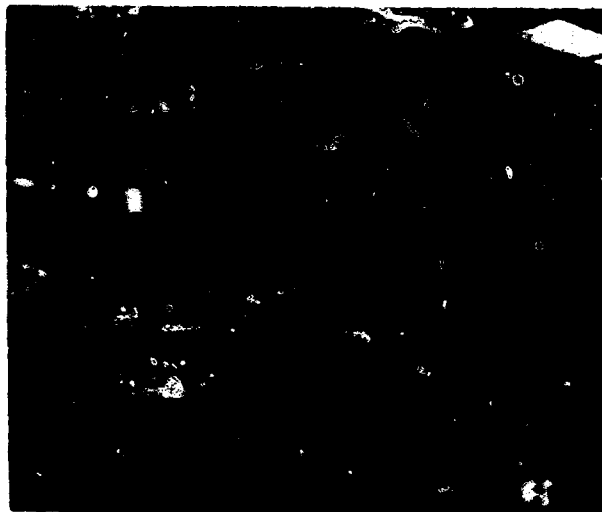


Figure 1. PSYCHOPHARMACOLOGICAL TEST CHAMBER

The responses of both animals to this task are shown schematically in figure 2. The time taken to respond to the sound increased for both animals during exposure to the ethylene glycol. However, this performance decrement which was initially acute, lessened as the exposure period continued.

The second task was more complicated, involving the adjustment of a Müller-Lyer illusion (figure 3) so that the two sections of the line were of equal length within a permitted tolerance. The animal was presented the illusion with the left section at a fixed length. The animal then adjusted the length of the other section using a small wheel. After pressing a lever to indicate the adjustment was complete, a food reward was given if the adjustment was correct within tolerance (20 mm for one chimpanzee and 15 mm for the other).

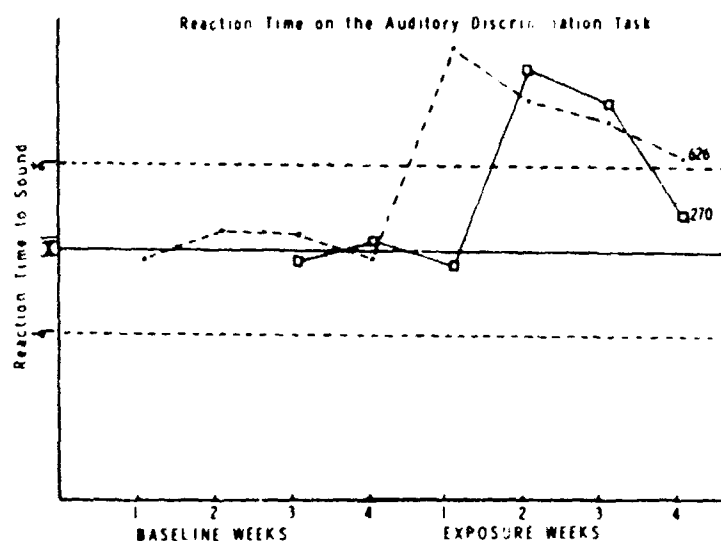


Figure 2

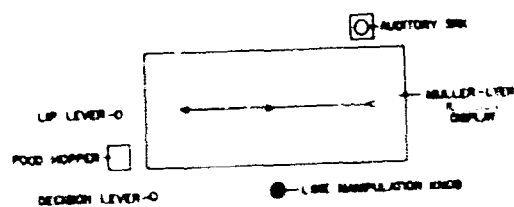


Figure 3. MULLER-LYER ILLUSION

One chimpanzee continued to maintain baseline performance levels during the exposure. The other chimpanzee exhibited a significant increase in deviation during the last two weeks of the experiment, as illustrated schematically in figure 4.

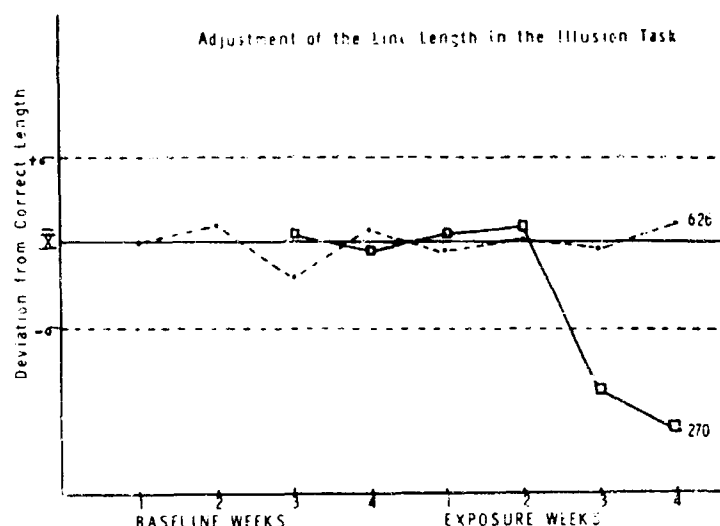


Figure 4

Hematologic values, which were determined on samples collected before and after ethylene glycol exposure, are shown in table II. There is an unexplained rise in the hemoglobin concentration and mean cell volume of the red cell in these animals. These animals did not exhibit the clinical signs associated with dehydration. Neither chimpanzee had a diarrhea. The chimpanzees appeared active and healthy when given physical examinations upon removal from the chamber. These observations are in agreement with the serum chemistry values for the two chimpanzees as given in table III.

TABLE II

## 28 DAY EXPOSURE TO ETHYLENE GLYCOL AEROSOL (HEMATOLOGY)

Index	ANIMAL NO. 270		ANIMAL NO. 626	
	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure
Hct (%)	40	46	42	45
Hgb (gm/100 ml)	13.1	15.0	14.3	15.1
MCV ( $\mu$ )	75.4	89.3	73.9	81.0
MCH ( $\mu$ )	24.7	29.1	25.2	27.2
MCHC (%)	32.7	32.6	34.0	33.8
WBC ( $10^3/\text{mm}^3$ )	11.8	19.5	11.9	7.1

TABLE III  
28 DAY EXPOSURE TO ETHYLENE GLYCOL AEROSOL (SERUM CHEMISTRY)

SERUM VALUES	ANIMAL NO. 270		ANIMAL NO. 626	
	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure
BUN (mg/100 ml)	16	18	8	10
Total Protein (g/100 ml)	7.0	6.8	7.5	7.1
SGOT (units)*	12	24	-	30
SGPT (units)*	22	31	-	18
Sodium (mEq/l)	142	138	142	142
Potassium (mEq/l)	4.72	4.37	3.91	4.41
Creatinine (mg/100 ml)	1.0	0.95	-	-
Alkaline Phosphatase (units)**	7.0	7.0	-	-

\* Sigma-Frankel

\*\* Bodansky

The only indication from clinical tests that the ethylene glycol exposure adversely affected these two chimpanzees was the results of the test for urine concentrating ability. Table IV lists the serum and urine osmolality, before and after glycol exposure. There is a decrease in the ability of the kidney to concentrate urine following the exposure. This is a condition commonly associated with disfunction of the distal tubules of the kidney. Other renal function tests which were performed on chimpanzee No. 626 were within normal limits - inulin clearance 1.9 ml/kg/min; PAH clearance 14.8 ml/kg/min; and PAH tubular maximum excretion 2.6 mg/kg/min. Examination of postexposure kidney biopsy specimen from chimpanzee No. 270 revealed oxalate crystals, while the histological preparations of material from chimpanzee No. 626 did not show any crystals.

To have a more realistic evaluation of the effects of ethylene glycol in the space-cabin environment, the following experiment was designed: four chimpanzees were placed in a Thomas Dome. The atmosphere of the dome was saturated with ethylene glycol vapor (256 mg/cubic meter at 70 C). The simulated altitude was 27,000 feet (5 psi, 68% oxygen, 32% nitrogen).



TABLE IV  
28 DAY EXPOSURE TO ETHYLENE GLYCOL AEROSOL  
(URINE CONCENTRATING ABILITY - 36 HOURS)

CHEMISTRY	ANIMAL NO. 270		ANIMAL NO. 626	
	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure
Serum Osmolality (mOs m/L)	270	274	280	296
Urine Osmolality (mOs m/L)	>1000	723	949	601
Ratio of Urine/Serum	3.7	2.6	3.4	2.1
Specific Gravity of Urine	1.035	1.005	1.025	1.016
Kidney Oxalate Crystals (biopsy)	no	yes	no	no
Inulin Clearance (ml/kg/min)			2.8	1.9
PAH Clearance (ml/kg/min)			16.5	14.8
PAH T <sub>m</sub> (mg/kg/min)			1.1	2.6
Inulin/PAH			0.17	0.13

A drop in the hemoglobin and hematocrit values occurred as shown in tables VA, VB, VC and VD. This was due to the large amounts of blood drawn. Approximately 80 ml of blood was drawn for each renal clearance test. Two tests were performed on each animal prior to exposure, one test at the midpoint of exposure, and one immediately after removal from the dome. Approximately 15 ml of blood was drawn weekly to follow the hematologic and serum chemistry values of each animal. Chimpanzee white blood cell counts fluctuate drastically in relationship to the state of excitement. However, all the animals showed a depression of total white count 2 weeks after the exposure period was completed. These are the first postexposure values given in tables VA, VB, VC and VD. The differential counts were normal.

The serum chemistry values of the chimpanzees exposed in the dome are given in tables VI A, VI B, VI C, VI D. The uric acid level of serum showed a slight rise in chimpanzee No. 255 (table VI A). The serum creatinine and blood urea nitrogen levels of all the chimpanzees remained normal throughout the experiment. The potassium levels increased mildly during exposure; however, this may be a function of altitude, rather than an effect of the glycol.

TABLE V A

28 DAY EXPOSURE TO ETHYLENE GLYCOL VAPOR (HEMATOLOGY) NO. 255

	<u>Baseline</u>		<u>Exposure</u>				<u>Post exposure</u>				
<u>Index</u>	<u>1</u>	<u>2</u>	<u>1wk</u>	<u>2wk</u>	<u>3wk</u>	<u>4wk</u>	<u>&lt;2 wk&gt;1wk</u>	<u>2wk</u>	<u>3wk</u>	<u>4wk</u>	
Hct (%)	49	38	41	41	35	36		40	43	43	45
Hgb (gm/100 ml)	16.3	12.3	12.8	13.2	11.4	11.7		13.9	13.8	14.1	15.1
MCV (cμ)	80.3	88.0	88.2	89.9	84.1	81.6		74.3	83.1	77.8	78.1
MCH (γγ)	26.7	28.7	27.5	28.9	27.4	26.5		25.8	26.7	25.5	26.2
MCHC (%)	33.3	32.6	31.2	32.2	32.6	32.5		34.8	32.1	32.8	33.6
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	16.1	11.3	15.1	6.7	10.9	8.3		6.5	17.0	8.6	27.0

TABLE V B

28 DAY EXPOSURE TO ETHYLENE GLYCOL VAPOR (HEMATOLOGY) NO. 665

Index	<u>Baseline</u>		<u>Exposure</u>				<u>Post-Exposure</u>
	1	2	1wk	2wk	3wk	4wk	
Hct (%)	46	38	35	38	40	41	No data due to death of animal
Hgb (gm/100 ml)	15.1	12.8	11.1	11.4	13.2	12.8	
MCV (cμ)	79.9	79.0	81.8	84.6	85.1	78.1	
MCH (γδ)	26.7	26.6	25.9	25.4	28.1	24.4	
MCHC (%)	33.5	33.7	31.7	30.0	33.0	31.2	
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	8.7	7.0	10.2	9.9	9.6	11.0	

TABLE V C

28 DAY EXPOSURE TO ETHYLENE GLYCOL VAPOR (HEMATOLOGY) NO. 675

Index	<u>Baseline</u>		<u>Exposure</u>				<u>Post exposure</u>			
	1	2	1wk	2wk	3wk	4wk <2wk>	1wk	2wk	3wk	4wk
Hct (%)	35	34	27		29	32	35	36	38	40
Hgb (gm/100 ml)	12.0	10.2	8.4		9.4	9.4	11.1	11.5	12.1	12.7
MCV (cμ)	75.9	77.1	74.8		69.0	72.4	66.5	71.3	72.1	74.1
MCH (δδ)	26.0	23.1	23.3		22.4	21.3	21.1	22.8	23.0	23.5
MCHC (%)	34.3	30.0	31.1		32.4	29.4	31.7	31.9	31.8	31.8
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	14.4	12.8	10.0		10.6	7.3	5.8	15.3	9.9	18.8

TABLE V D

28 DAY EXPOSURE TO ETHYLENE GLYCOL VAPOR (HEMATOLOGY) NO. 684

Index	<u>Baseline</u>		<u>Exposure</u>				<u>Post exposure</u>			
	1	2	1wk	2wk	3wk	4wk <2wk>	1wk	2wk	3wk	4wk
Hct (%)	36	27	33	34	32	32	34	38	39	42
Hgb (gm/100 ml)	11.1	8.8	9.9	11.4	10.2	9.6	10.5	11.5	12.5	14.1
MCV (cμ)	71.0	75.4	78.4	65.4	78.8	76.4	65.0	76.9	73.6	75.0
MCH (δδ)	21.9	24.6	23.7	21.9	25.1	22.9	20.1	23.3	23.6	25.2
MCHC (%)	30.8	32.6	30.0	33.5	31.9	30.0	30.9	30.3	32.0	33.6
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	18.3	19.1	10.7	10.3	18.0	10.1	7.1	21.0	15.3	17.8

TABLE VI A

28 DAY EXPOSURE TO ETHYLENE GLYCOL VAPOR (SERUM CHEMISTRY) NO. 255

Chemistry	Baseline	Exposure				Post exposure		
		1wk	2wk	3wk	4wk	<2wk> 1wk	2wk	3wk
Sodium (meq/l)	141	139	140	143	138	139	146	143
Potassium (meq/l)	3.8	3.7	3.5	4.3	3.7	4.0	4.7	4.9
BUN (mg/100 ml)	10	5	7	10	8	11	10	11
Total Protein (gm/100 ml)	6.9	7.8	7.1	7.9	7.7	7.3	7.1	7.7
Creatinine (mg/100 ml)	1.0	0.8	0.5	0.8	0.6	0.7	0.9	-
Uric Acid (mg/100 ml)	1.8	4.2	3.8	4.0	3.0	2.2	-	-
SGOT (units)*	-	15	10	16	15	-	-	-

\*Sigma-Frankel

TABLE VI B

28 DAY EXPOSURE TO ETHYLENE GLYCOL VAPOR (SERUM CHEMISTRY) NO. 665

Chemistry	Baseline	Exposure				Post exposure		
		1wk	2wk	3wk	4wk	<2wk> 1wk	2wk	3wk
Sodium (meq/l)	140	137	140	141	140	No data due to death of the animal		
Potassium (meq/l)	4.6	3.8	4.0	4.5	4.0			
BUN (mg/100 ml)	11	7	9	8	5			
Total Protein (gm/100 ml)	7.1	6.8	6.9	7.8	7.4			
Creatinine (mg/100 ml)	1.0	0.5	0.4	0.8	0.7			
Uric Acid (mg/100 ml)	-	3.8	4.0	4.5	4.0			
SGOT (units)*	-	15	10	15	15			

\*Sigma-Frankel

TABLE VI C

28 DAY EXPOSURE TO ETHYLENE GLYCOL VAPOR (SERUM CHEMISTRY) NO. 675

Chemistry	Baseline	Exposure				Post exposure		
		1wk	2wk	3wk	4wk	<2wk> 1wk	2wk	3wk
Sodium (meq/l)	137	138		140	138	136	143	138
Potassium (meq/l)	3.1	3.1		4.4	3.4	4.0	4.5	4.7
BUN (mg/100 ml)	11	7		9	6	11	12	10
Total Protein (gm/100 ml)	6.7	7.1		8.1	7.7	6.6	6.9	7.1
Creatinine (mg/100 ml)	-	0.6		0.6	0.6	0.8	0.7	-
Uric Acid (mg/100 ml)	-	2.7		3.8	3.8	2.6	-	-
SGOT (units)*	-	15		15	15	-	-	-

\*Sigma-Frankel

TABLE VI D

28 DAY EXPOSURE TO ETHYLENE GLYCOL VAPOR (SERUM CHEMISTRY) NO. 684

Chemistry	Baseline	Exposure				Post exposure		
		1wk	2wk	3wk	4wk	2wk 1wk	2wk	3wk
Sodium (meq/l)	143	139	144	142	138	141	151	139
Potassium (meq/l)	2.2	2.6	3.1	2.4	2.8	4.6 <sup>†</sup>	4.0	3.7
BUN (mg/100 ml)	9	7	5	10	7	13	10	12
Total Protein (gm/100 ml)	7.3	7.6	6.5	7.9	7.6	7.4	7.8	7.9
Creatinine (mg/100 ml)	-	0.5	0.4	0.5	0.5	0.7	-	-
Uric Acid (mg/100 ml)	-	2.8	2.5	2.8	2.8	3.1	-	-
SGOT (units)*	-	15	15	18	15	-	-	-

\*Sigma-Frankel

TABLE VII  
RENAL CLEARANCE STUDIES

Clearance	Baseline		Exposure	
	1	2	14 days	28 days
<b>255</b>				
Inulin (ml/kg/min)	2.3	2.4	2.5	2.8
PAH (ml/kg/min)	15.5	9.8	15.1	14.3
T <sub>m</sub> PAH (mg/kg/min)	1.9	2.4	2.6	2.1
Inulin/PAH	0.22	0.20	0.24	0.19
<b>665</b>				
Inulin (ml/kg/min)	1.8	2.2		2.2
PAH (ml/kg/min)	8.1	7.2		8.9
T <sub>m</sub> PAH (mg/kg/min)	1.9	2.1		2.1
Inulin/PAH	0.26	0.24		0.26
<b>675</b>				
Inulin (ml/kg/min)	3.2	1.8	2.4	3.1
PAH (ml/kg/min)	13.1	10.9	10.7	11.4
T <sub>m</sub> PAH (mg/kg/min)	2.8	3.4	2.8	2.3
Inulin/PAH	0.31	0.19	0.19	0.26
<b>684</b>				
Inulin (ml/kg/min)	2.6	3.5	2.8	3.8
PAH (ml/kg/min)	11.3	17.2	19.7	17.5
T <sub>m</sub> PAH (mg/kg/min)	3.7	3.0	4.0	2.6
Inulin/PAH	0.23	0.17	0.15	0.20
<hr/>				
	Mean Values - Baseline		Standard Deviation	0.01 Confidence limit
Inulin (ml/kg/min)	2.4		0.9	1.3 - 3.5
PAH (ml/kg/min)	11.4		2.9	7.7 - 15.1
T <sub>m</sub> PAH (mg/kg/min)	2.6		0.6	1.8 - 3.3
Inulin/PAH	0.23		0.02	0.18 - 0.27

The results of the renal clearance studies are summarized in table VII. The baseline values differ slightly from those given by Hamlin (1964) for immature chimpanzees. There was no significant difference between the baseline values and those found after 14 and 28 day exposure. Chimpanzee No. 665 was not tested in the dome. It is unfortunate that the test for kidney urine concentrating ability could not be done on these animals.

Sykowski (1951) and Esparza (1967) reported corneal damage due to ethylene glycol. Examination of the eyes of these six chimpanzees for evidence of cornea erosion gave negative findings in all cases.

### CONCLUSIONS

Inhalation of ethylene glycol at the level of 256 mg/m<sup>3</sup> for periods of 28 days did not appear to be seriously harmful. A small decrease in urine concentrating ability indicated slight impairment of distal tubular function. Whether this condition would become more serious with a longer period of exposure is unknown.

Serious behavioral disturbances, such as have been observed in both man and the chimpanzee following ingestion or injection of ethylene glycol, did not occur during the periods of glycol inhalation. When ethylene glycol is ingested or given by intravenous injection, ataxia, vomiting and various other disfunctions of the nervous system may result. Continual metabolism of the glycol as it is taken up may prevent the build-up of a sufficient quantity of glycol to affect the central nervous system.

Although the present study was done with chimpanzees, it seems appropriate to draw certain conclusions about man. It is improbable that man could inhale a sufficient amount of ethylene glycol to be harmful, at least during a 28 day period. Pre-existing pathology might be aggravated by such exposure. Oxalate crystals could seed the development of kidney stones. The possibility is not great during short duration exposures, but could be a problem for chronic exposure.

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## DISCUSSION

MR. BATTISTA (Arthur D. Little, Inc., Cambridge): Have you done any of the experiments in tracheotomized animals?

CAPTAIN FELTS (Aeromedical Research Laboratory): No, we haven't.

MR. BATTISTA: With a 5 micron particle, you might be running into a situation where the dose that you're actually getting systemically may be a lot different from what you have presented to the animal. The next question is have you checked ethylene glycol as far as its effect on cilia?

CAPTAIN FELTS: No, we haven't.

DR. THOMAS: First of all, for your question back there, we did not introduce it as an aerosol. It was all ethylene glycol vapor in the Dome experiment.

CAPTAIN FELTS: Yes. But in our performance chamber at Holloman it was introduced as an aerosol.

DR. THOMAS: But at Wright-Patterson it was as a vapor. You wouldn't have a problem in the dome of the 5 micron particles. I want to highly commend Capt. Felts for doing this work. It took a lot of doing and believe me, she was flying the chambers, doing the kidney function studies in there, which added a touch of beauty to our Christmas holidays. Besides that it was the first time we had chimps in the domes. One question, did you taste the sweetish taste when you were working up there, five hours sometimes?

CAPTAIN FELTS: You could taste a little sweetness on the lips, but it wasn't an unpleasant thing to make me want to get out.

DR. THOMAS: There's a puzzle here because we had saturated vapors and people were able to work in it, highly efficiently.

DR. PFITZER: I wonder if you could correlate for us the concentrations in your experiment with those in the previous human experiment. I'm a little confused by the parts per million and your milligrams per cubic meter, how comparable they are.

CAPTAIN FELTS: Two hundred and fifty-six milligrams per cubic meter is approximately 200 parts per million, I believe.

MR. VERNOT: It's 100 parts per million.

CAPTAIN FELTS: At altitude it gets complicated to work with parts per million I'm told, so we stuck to milligrams per cubic meter.

DR. SLONIM: You spoke, or you showed some results with the distal tubules. Did you do, or rule out, any effects on glomerular filtration by the creatinine clearance although you had just one value for creatinine? You didn't have a filtration rate.

CAPTAIN FELTS: The inulin clearances, since they were normal, should have ruled out any glomerular problems. Really, what you're measuring when you do inulin clearance is glomerular filtration rate.

DR. CAMPBELL (U.S. Public Health Service, Cincinnati): This is more a question of a chemist than of Capt. Felts. I was wondering if in a 50% or greater humidity situation, saturated vapor atmosphere would produce any particulates or aerosol?

MR. VERNOT: No, we saw no aerosol. Of course we introduced the ethylene glycol through a heating system. We vaporized the ethylene glycol directly into the chamber without putting in water first. The relative humidity in the dome is approximately 50% and we saw no aerosol at approximate saturated vapor pressure of ethylene glycol.

DR. CAMPBELL: Visually?

MR. VERNOT: That's right. As a matter of fact, we did a rough Tyndall test. We turned off all the lights, and turned the flashlight on the side to see if we could see any scattering, and we saw none.

DR. CAMPBELL: Wouldn't this depend pretty much on particle size, if you generate large particles?

MR. VERNOT: We generated the vapor; we didn't use aerosol.

CAPTAIN FELTS: Let me make a statement about our performance chambers. This was a continuous flow system which carried the aerosol in and when it came in it was immediately swept through the chamber and back out, so that it was an aerosol.

MR. WANDS: Thank you, that's an important feature. Now, is this the same material used in the spacecraft, or does the spacecraft heat exchanger carry the anti-oxidant?

CAPTAIN FELTS: Dr. Harris will have to answer that. I don't see him here, but it's my understanding that they used the analyzed reagent. This is what I wouldn't say is an absolute positive fact.

DR. JACOBSON (Tulane University): I'd like to speculate for a minute on the question that Dr. Thomas raised. I believe you said you could not taste the saturated vapor but the aerosol has a taste. Did I understand you correctly?

CAPTAIN FELTS: I said I could taste the vapor on my lips after I had been in the dome for several hours, but it did not give me the same reaction as the prisoners had with the aerosol.

DR. JACOBSON: What I was thinking was that on the matter of absorption of aerosols, many of the statements made of the efficiency of absorption applying to low particle size apply to non-absorbable aerosols. In other words, we're talking there about the alveolar absorption. Now, if you have an aerosol which is water soluble and is absorbable in tissues other than the alveoli, then there is a higher efficiency of absorption in the upper respiratory tract with larger aerosol particles. Thus, a high particle size water soluble aerosol may be absorbed in the upper respiratory tract, even the throat and mouth, leading to a taste; whereas the vapor may not be absorbed until it gets to the lower respiratory tract. That's speculative.

DR. THOMAS: The main difference in the human reactions to this substance is that we didn't have the urge to get out of the chamber, while the prisoners would not stay voluntarily in the room if the concentration was over 70 to 100 ppm.

DR. COULSTON: Somewhere about that.

DR. PFITZER: I'm still confused about these concentrations. Dr. Coulston defined the 75 ppm as a fog in their chambers, and you have indicated that your saturated vapor was equivalent to about a 100 parts per million, and I'm having a little difficulty reconciling these. I'm also interested in the experiment you did at Holloman with the aerosol at the 200 plus milligrams per cubic meter. Was this a fog?

CAPTAIN FELTS: Yes, it was. It didn't seem to bother the chimpanzees.

DR. COULSTON: We measured in our human study and in our studies with monkeys at Albany Medical College, the number of micrograms per liter of air and now you can convert that any way you want. So, when we're talking in the prison studies we say 50 micrograms per liter or if you want to convert it 50 milligrams per cubic meter, you're welcome to it. I don't care what you call it. These are the figures we used. In our own naivete in our own shop we stick to micrograms per liter of air because it is convenient. When we publish this we'll convert it into the customary jargon.

MR. WANDS: Then the numbers that were cited were micrograms per liter rather than ppm?

DR. COULSTON: Cited where?

MR. WANDS: This morning, for the human exposures. No effect at 25 ppm and no effect at 25 microgram per liter.

DR. COULSTON: I believe 25 micrograms which makes . . . .

MR. WANDS: That should help clarify the problem.

DR. MAC EWEN (Systemed Corporation): Yes, that's considerably less. Twenty-five micrograms per liter is approximately 5 parts per million equivalent.

This taste bit, I might make one comment. In the aerosol generation, taste was on the tongue where the aerosol droplets actually collected in the mouth or could be tasted, physically. In the saturated vapor exposures, the taste is that which is collected on the lips and skin from the licking. You get a secondary effect of taste only after you lick your lips.

CAPTAIN FELTS: I see Dr. Harris is here now. Perhaps he can answer the question. The question was, Dr. Harris, was the glycol that was used in the capsule, was it analyzed reagent or was it commercial grade with the anti-oxidants in it?

DR. HARRIS: The stuff that was used in the capsule has anti-oxidants and it also has some other stuff in it. There are two additives. One was sodium mercaptothiazole and the other was triethanolamine. It's definitely not the reagent grade.

EFFECT OF HYPEROXIA ON THE SURVIVAL OF RED BLOOD  
CELLS IN THE RAT USING  $^{14}\text{C}$  TECHNIQUE

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INTRODUCTION

The question of whether oxygen at pressures greater than that found in air at sea level can cause the breakdown of red blood cells has been investigated by a number of workers, and it is now fairly well accepted that oxygen at partial pressures greater than 1 atmosphere can indeed cause an in vivo peroxidative hemolysis, particularly in vitamin E deficient animals (Raihi, 1955; Kann, 1964). On the other hand, attempts to demonstrate hyperoxia-induced hemolysis at oxygen pressures below 1 atmosphere without equivocation have not been forthcoming. As it turns out, one of the major physiologic changes reported in the Gemini manned space flights of 3 days or longer was a decrease in the red cell mass on the order of 10% (Fischer, 1967).

Three potential causative factors which have been considered for this phenomenon are:

1. Hypodynamia
2. Weightlessness
3. The pure hyperoxic space cabin atmosphere.

Our particular interest has been directed toward obtaining a conclusive answer to the third possibility. That is, does mild hyperoxia cause the hemolysis of red blood cells in vivo?

## METHODS AND MATERIALS

The primary technique used in this study was developed by Landaw (1966, 1969). It is a cohort labeling technique, based on the fact that when red blood cells are destroyed in the body and the hemoglobin is degraded, the heme ring is opened at the alpha-methene bridge carbon atom with the oxidation of this carbon to carbon monoxide. Furthermore, the unique source of the methene bridge carbons of heme is the 2-carbon of glycine (see figure 1). Therefore, for every mole of heme degraded, 1 mole of CO is produced and exhaled. Figure 1 demonstrates also that if  $^{14}\text{C}$  glycine labeled in the 2 position is administered to an animal, it is rapidly incorporated into hemoglobin heme of newly forming red blood cells. The glycine also labels nonhemoglobin hemes of the cytochromes and other heme constituents and labels protein as well. The non-hemoglobin hemes have a very rapid half-life. The glycine-labeled protein turnover is very slow in the rat, being on the order of 50-100 days.

## PATH OF CARBON MONOXIDE FORMATION

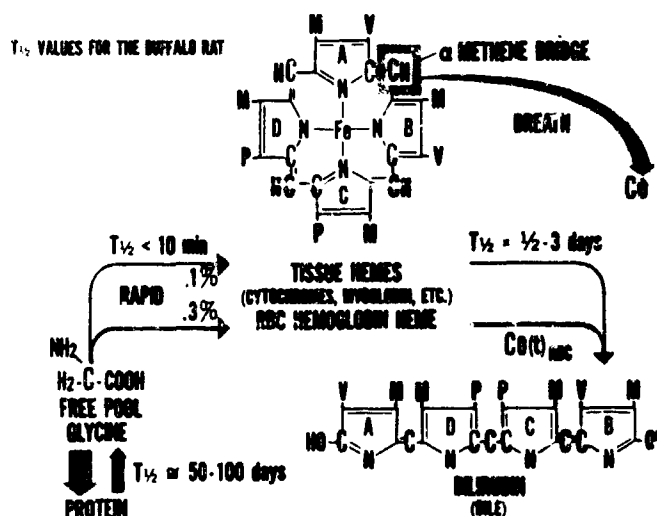


Figure 1. PATH OF CARBON MONOXIDE FORMATION.

Figure 2 shows the output of  $^{14}\text{C}$  from a normal Buffalo rat and illustrates how survival parameters of red blood cells can be derived. The function  $F(\text{CO})_{\text{total}}$  describes the  $^{14}\text{C}$  data.  $F(A)$  and  $F(B)$  represent the  $^{14}\text{C}$  generated from nonhemoglobin hemes and recycling of 2- $^{14}\text{C}$ -glycine from protein into heme. If these are subtracted from  $F(\text{CO})_{\text{total}}$  then  $F(\text{CO})_{\text{RBC}}$  which describes  $^{14}\text{C}$  output attributable to RBC degradation, is obtained. From this function the survival parameters of the population of RBC in question can be extracted. As one would expect the  $^{14}\text{C}$  output is in fact describable as a function of the circulating radioactive hemoglobin content.

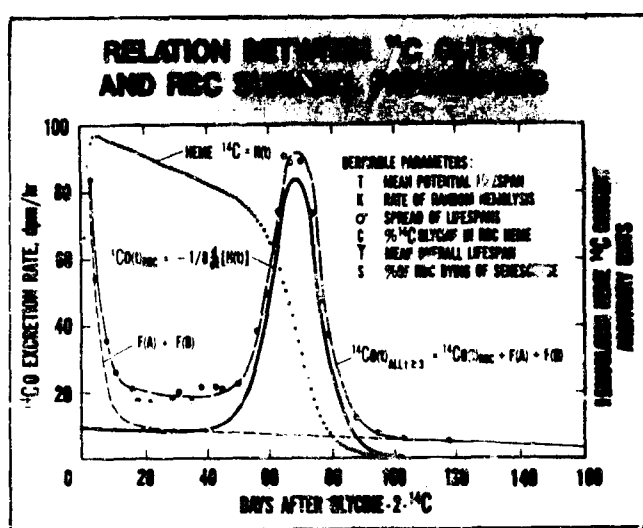


Figure 2. RELATION BETWEEN  $^{14}\text{C}$  OUTPUT AND RBC SURVIVAL PARAMETERS.

The parameters of interest which are obtainable in this fashion are:

- $T$  - mean potential lifespan
- $k$  - the rate of random hemolysis
- $\sigma$  - the spread of lifespans about  $T$
- $\bar{T}$  - the mean overall lifespan
- $C$  - the percent of the injected glycine dose incorporated into RBC heme.

The capsule system for exposing rats continually to oxygen for long periods is shown in figure 3 (Quattrone, 1966). Also shown is the train for  $^{14}\text{C}$  collection (Landaw, 1966).





Figure 3. EXPOSURE SYSTEM AND  $^{14}\text{C}$  CO COLLECTION TRAIN.

#### RESULTS AND DISCUSSION

In our studies on hyperoxia we wanted to know:

1. Does oxygen affect RBC as they are being formed?
2. Does oxygen affect RBC formed under normal circumstances? If so, does the effect depend on the age of the RBC at the time of the initial exposure?
3. In either case, should there be an effect, is it dose related? That is, is there a threshold for the effect, and does it become more pronounced with increasing  $\text{PO}_2$ ?

In our initial studies the following groups were studied:

1. RBC cohorts labeled in normal rats which were placed in oxygen 14 days later at 197, 258, or 450 torr.  $^{14}\text{C}$  output was determined under these conditions for 80 days.
2. Rats placed in oxygen at 197, 258, or 450 torr for 2 weeks and then a cohort was labeled. The rats were placed in their respective oxygen environments, and  $^{14}\text{C}$  was measured for 92 days.
3. Control rats labeled at the time of the other rats and  $^{14}\text{C}$  measured for 92 days in an air environment under sea-level conditions.

The results are listed in table I. The most remarkable finding is that the mean potential lifespan of young RBC exposed to pure oxygen for extended periods is shortened significantly. As we can see, all groups exposed to the oxygen when the labeled cells were 14 days old showed this effect. Thus, even those exposed to 197 torr, which is normoxic with respect to the alveolar partial pressure, are affected. One interpretation that can be made from this is that pure oxygen at a given partial pressure has a greater physiologic effect than in the presence of a diluent. That this may be the case is also indicated by the combined results of the Gemini and Apollo flights with respect to the red cell mass changes, for it appears that only when astronauts were exposed to pure oxygen for some time were decreases in the red cell mass noted (Fischer, 1969).

TABLE I

EFFECT OF O<sub>2</sub> PRESSURES ON PREFORMED AND FORMING RED BLOOD CELLS

		Mean Potential Life Span	Random Hemolysis	Spread of Life Spans	Fraction Incorporated
N		T (Days)	k (% day)	$\sigma$ (days)	C (% of dose)
	(4) Control	69.2 $\pm$ .9	.69 $\pm$ .08	6.4 $\pm$ .7	.265 $\pm$ .031
AIR LABELED	(4) 197 torr	66.0 $\pm$ .8	.65 $\pm$ .07	8.0 $\pm$ .2	.342 $\pm$ .024
	(4) 258 torr	65.9 $\pm$ 1.1	.62 $\pm$ .03	7.8 $\pm$ .3	.240 $\pm$ .021
	(4) 450 torr	63.8 $\pm$ .3	.77 $\pm$ .05	7.6 $\pm$ .3	.269 $\pm$ .015
	(12) Pooled	65.2 $\pm$ .5	.68 $\pm$ .03	7.8 $\pm$ .2	.290 $\pm$ .015
OXYGEN LABELED	(3) 197 torr	68.4 $\pm$ .4	.83 $\pm$ .09	8.1 $\pm$ .1	.215 $\pm$ .021
	(4) 258 torr	68.8 $\pm$ .2	.58 $\pm$ .03	7.1 $\pm$ .8	.202 $\pm$ .017
	(5) 450 torr	66.7 $\pm$ 1.0	.83 $\pm$ .06	6.4 $\pm$ .3	.161 $\pm$ .014
	(12) Pooled	68.0 $\pm$ .4	.76 $\pm$ .04	7.2 $\pm$ .3	.192 $\pm$ .011

Italic type indicates a significant difference from control.  $P \leq 0.05$

The control mean potential lifespan was 69.2 days. In pure oxygen the value was 66.0 days in 197 torr, 65.9 days in 258 torr, and 63.8 days in 450 torr. This trend suggests a dose effect.

There was some effect on the spread of lifespans about the mean,  $\sigma$ , and it was significant for the pooled data. This indicates that the effect of oxygen on the mean potential lifespan is a function of the normally expected mean potential lifespan. There was no effect on the rate of random hemolysis. The fractional incorporation of glycine was normal in all instances, as is to be expected since the rats were injected with the glycine while still under normal conditions.

In view of the effect of oxygen on the mean potential lifespan of circulating red blood cells, the lack of any effect of oxygen on forming red blood cells (oxygen labeled group, table I) is rather surprising. The percent random hemolysis and the spread of lifespans appears normal in spite of continuing exposure to oxygen. One possibility is that RBC formed during hyperoxic exposure are more resistant to the hyperoxia. If this is the case, one might expect that such cells would have a greater longevity under normal conditions where the oxidative stress is lessened.

What we have seen thus far is an effect of long-term exposure to oxygen on formed red blood cells. In another set of experiments we wanted to determine the effect of acute exposure on older RBC nearing the end of their normal lifespan. In this case we used an  $O_2$  pressure of 600 torr. The results of this experiment are illustrated in figure 4. The rats were labeled on Day 0 and placed in capsules in air on Day 40. On Day 55, after the senescence process had started, the rats were switched over to oxygen. It can be seen that there appears to be an almost immediate increase in the output of  $^{14}CO$  indicating an increased rate of hemolysis of the older cells. This spike represents about 11% of the cells potentially at risk on Day 55. Thus it appears that  $O_2$  can indeed cause the lysis of red blood cells and this effect appears to have some relationship to the age of the cells at the time of the initial exposure.

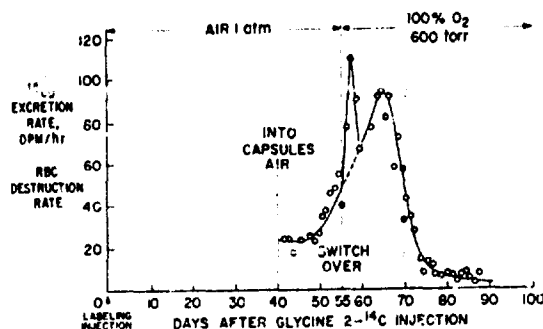


Figure 4. EFFECT OF OXYGEN AT 600 TORR ON DESTRUCTION RATE OF SENESCENT RED BLOOD CELLS.

The normalization of the senescence process after 3-5 days and the lack of any effect of continuing oxygen exposure on the survival parameters of labeled cohorts formed after 2 weeks of oxygen exposure could be interpreted to mean that the oxygen was not directly affecting RBC. In order to obtain a direct answer to this question the experiments listed in table II were performed. Normal rats were kept in capsules in air at sea level or in oxygen at 600 torr for 5 days since earlier experiments indicated that this period of time is required for the maximal effects of oxygen to be expressed. The rats were then infused with 2.0 ml of washed RBC from rats labeled with 2-<sup>14</sup>C-glycine 53 days previously. Thus these cells were already beginning to undergo senescence. The rats were then split into 4 groups and immediately placed in the respective environments indicated. The formation of <sup>14</sup>CO was then measured. From these results it is clear that regardless of the previous condition of the rat, exposure to oxygen increases the breakdown of red blood cells and is measurable within the first 5 hours of exposure. Conversely, rats with an immediate prior history of exposure to oxygen do not respond differently from normal animals either to oxygen or to air. It is concluded from these findings that the effect of oxygen on the RBC is a direct one, and no residual effect indicative of tissue changes is demonstrable.

TABLE II

EFFECT OF PRECEDING AND IMMEDIATE ENVIRONMENTAL CONDITIONS  
ON <sup>14</sup>CO OUTPUT FROM INFUSED RBC LABELED 53 DAYS PREVIOUSLY

Initial Exposure Condition (N)	Immediate Postinfusion Exposure Conditions	<sup>14</sup> CO Output dmp/hr 1st 5 Hrs Postinfusion	Significantly Different From
1. Air, 760 torr (6)	Air, 760 torr	24.6±1.5*	2, 4
2. Air, 760 torr (4)	O <sub>2</sub> , 600 torr	39.3±3.0	1, 3
3. O <sub>2</sub> , 600 torr (6)	Air, 760 torr	18.9±2.2	2, 4
4. O <sub>2</sub> , 600 torr (6)	O <sub>2</sub> , 600 torr	34.8±2.7	1, 3

\*S.E.M.

Mature male Buffalo rats were exposed to air at sea-level or to pure oxygen at 600 torr for 5 days. Then, 2.0 ml of pooled and washed RBC from donor rats which had been injected with 2-<sup>14</sup>C-glycine 53 days, previously were infused into each rat. The rats were immediately placed in the indicated postinfusion environment and 5-hr samples of <sup>14</sup>CO were collected.

In conclusion, mild hyperoxia causes hemolysis of rat red blood cells in vivo, and shortens the lifespan of cells exposed for prolonged periods.

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## DISCUSSION

MAJOR THEODORE: I just have a few questions. First, have you done iron kinetic studies or iron tagging to see if these match up with your data as well as for as survivability, seeing where the cells are going, whether they are having intravascular hemolysis, whether the cells are damaged and being picked up by the spleen, or are they actually being destroyed in the marrow?

DR. LEON: No, we haven't done iron kinetic studies. Dr. Winchell, who is a foremost expert on iron kinetics has done the iron kinetics on the aquanauts in the Sea Lab III, and in this case, there was an inert gas present even though the environment was hyperoxic, and he found no changes in the iron kinetics. We haven't done the things you suggest. We have done other types of experiments with osmotic fragility and red blood cell enzyme changes where the data is in line with what we found here. The osmotic fragility studies indicate that you have a younger population which would follow if you knock off the older ones, and the enzyme studies done by Dr. Sabin in our lab indicate the same thing. Within three days you get an increase in G6PD and hexokinase, and the only way you can get an increase is to have a younger population.

MAJOR THEODORE: Is this due to increased marrow activity or does the marrow not respond then?

DR. LEON: No, apparently--

MAJOR THEODORE: Iron uptakes would be accelerated under points of intravascular hemolysis.

DR. LEON: Well, all evidence seems to indicate that there is a suppression of erythropoiesis that was indicated by our decreased uptake of the glycine and other people in the past have done work which supports the idea that hyperoxic exposures suppress erythropoiesis. In view of that, if you accept that as being true, the only way to get an increase in an enzyme in a non-nucleated cell like the red blood cell, or the only way that you can get an increased resistance to osmotic fragility would be to have a younger population and the only way that you can get a younger population under these conditions, at least the only way that I can imagine, is to knock off the older ones.

MAJOR THEODORE: That's true. This is a beautiful technique using the glycine tag in the two position evolving carbon monoxide, but I was wondering here in the methodology, how do you correct a CO washout effect? By this I mean over this period of 60 days you're measuring peak CO output. During that time you may have some

CO leak that's now bound to hemoglobin--not incorporated, but bound--and I think some of the peaks you got initially upon exposure to oxygen may well be just the competitive release of carbon monoxide from the heme which is in actual competition with oxygen for the same site. I think this is a moot point. Out of curiosity I was wondering if you were able to correct for this CO output versus that which is being evolved when you break down your heme.

DR. LEON: Well, that bothered me at first when I first was introduced to this technique, because you always hear that carbon monoxide binds to hemoglobin two to three hundred times greater than oxygen. This is true, but, in fact, it's in dynamic equilibrium. For example, we have done some early studies, early peak studies, where you inject the glycine and immediately after injecting the glycine you start to take your carbon monoxide samples, and from this you can determine turnover rates of your cytochromes and you can determine the quantity of ineffective erythropoiesis and you can determine the extent at which little driplets of hemoglobin come out of the retics on the mitochondrion, and so forth. What you see is that you inject the glycine, and within seconds you're getting your carbon monoxide out and, in fact, the peak of the carbon monoxide output from the cytochromes is at a half hour after the injection. So, you're right that it's bound tightly to the hemoglobin, but it's just like it comes in and pushes it out. It goes real fast. It becomes mixed in rapidly, within seconds, and it's out in proportion to its quantity, very rapidly.

MAJOR THEODORE: I just have one more question and then I'll quit. Has anyone looked at the marrow, per se, by electron microscopy with those techniques where one wants to look at the red cell mitochondria, since hemoglobin synthesis in the red cell precursors occurs at these sites? When you're talking about things like sideroblastic anemias, etc. where they demonstrate definite mitochondrial lesions, I was wondering if this type of model or analogy was looked at from this basis, since you have presented evidence that there may be some abnormality of incorporation. If one wants to stretch his imagination a bit one could look at this as a sideroblastic type of problem and therefore maybe looking at the mitochondria and seeing if there are changes, but this is in the marrow cells because as the cells mature they lose this. This is just another point. I'm not refuting what you're saying; I'm saying some things that maybe one could look at--

DR. LEON: I don't think--I wouldn't call it abnormal; I would just call it decreased.

MAJOR THEODORE: That's abnormal. You're losing 20% of your red cell mass, if I understand the data on the astronauts. I think that's significant.

DR. LEON: Well, it's significant, but it may just be physiological. I like to differentiate between a physiological mechanism and an abnormal mechanism. You can accelerate and decelerate a physiological mechanism and still not be abnormal.

MAJOR THEODORE: That's because our people have recovered.

DR. LEON: For example, if you go to high altitude you've got an accelerated production of red blood cells, and this certainly isn't abnormal; it's a normal reaction. That's the point I was making, but I don't know that anybody has looked at the red blood cells. I think there are people who are very interested in doing it and maybe some of the electron microscopists in the group might be able to comment on that.

CAPTAIN CHIKOS: The only question I had was you are demonstrating a decrease in the red cell population, and when one uses a term hemolysis you usually imply a pathological state of intravascular lysis of red blood cells, and I think it would be more appropriate to say that you're just having a decreased survival time for whatever cause. It might be just accelerated destruction by the spleen in this case, or some other physiological mechanism.

DR. LEON: Yes, I don't disagree with you. Actually, this technique is extremely sensitive and it has been difficult for us to actually demonstrate, in these particular cases, a decreased red cell mass, although we are going to try to do this next.

DR. CAMPBELL: I was wondering if anyone could comment on the possible formation and effects by peroxides in these situations, and also, I might disagree with the allusion made that the only mechanism for red cell fragility might be younger cells, because I should think that possible direct effects on red cell membranes could also result in this without there being an effect on the age of the cell.

DR. LEON: Well, with respect to that latter point, I would say that I don't know of any instance where some toxic or peroxidative agent increases the resistance of red blood cells to osmotic fragility. It usually decreases their resistance, so-- you're right. This is a weak point, and actually, we are working on the osmotic fragility quite heavily, trying to show that the osmotic fragility changes when the osmotic fragility decreases, that it indicates a younger population. We haven't really proven that to our satisfaction as yet.

MAJOR CASEY (School of Aerospace Medicine): I didn't get how you ruled out a stimulatory effect on the RE system or spleen, that it was a direct effect on the red cells themselves.

DR. LEON: Well, the rats that were exposed to oxygen at 600 torr for 5 days prior to the infusion of the 53-day old cells, when they were placed into air, they actually had a lower breakdown of red blood cells, as indicated by the carbon monoxide output, than the normals. The red blood cells that were infused, the ones that were 53 days old, came from normal rats, that is, these rats had just been kept in air, so these were untreated red blood cells other than the fact that they were radioactive. These rats had been in oxygen for five days and then they were immediately injected with 2 mls of the 53-day old red blood cells and they were put in air right after and the carbon monoxide output was determined. The value was actually lower than in the normal control, but statistically, they were the same, so that you can say that the breakdown of red blood cells in this group which had been conditioned to the oxygen was no different than normal controls. So there is no indication from this that the RE system had been stimulated. If it had been stimulated, it was an on-off stimulation.



MAJOR CASEY: In this circumstance, don't you get blood mismatches in rats as well? How much hemolysis was induced here by antibodies? You're taking blood from one animal and putting it in another.

DR. LEON: These are pretty homogenous rats.

MAJOR CASEY: I don't know how homogenous rats are; that's what I'm asking.

DR. LEON: Nevertheless, this experiment is a controlled experiment, and you'd see it. This is your control, and part of this output here is actually due to a breakdown of some damaged red blood cells, taking them up in the syringe and infusing them, so this would form a control on that question that you raise.

This is a normal control, 24.6. This was the air that was placed in the oxygen. It's 39.3 and it is significantly higher. These are the ones that had been in oxygen and were put into air and they're 18.9. They're not significantly different from the controls, but they are significantly lower than this group here, and this is 34.8. These were in oxygen initially and were put in oxygen subsequently, so no matter what the prior condition was, if they were put into oxygen they had high outputs of carbon monoxide, and no matter what the prior condition was, if they were put into air they had low carbon monoxide outputs.

DR. WEIBEL: Well, I don't want to take too much time. I'm going to put some of the comments I wanted to make in my paper this afternoon where you shall see that we have found essentially data on the changes in the blood at one atmosphere of pure oxygen in rats which are quite in keeping with the data that Dr. Leon showed. Actually, I'm very happy about his findings because as you know they are somewhat different from what other people have said. We have felt a little uneasy about that. I was a little worried about your comment that the red cell erythropoiesis would be depressed. Is this really supported by evidence? Because we found that there was no decrease in red cell mass; on the contrary, you will see that hemoglobin and hematocrit increased after 48 and 60 hours in pure oxygen. Of course, you may have the question that this is probably due to fluid loss, but still for two days when you get no change and where you get no edema formation, you have very stable hemoglobin and hematocrit in these rats. Well, I have just one question. What was the time in your second experiment until this extra peak occurred? This was a little compressed on the slide, you know. The one you gave after 55 days of pure oxygen. What was the time of the peak?

DR. LEON: We put them into oxygen and immediately started the determination and we had to take a five-hour determination because there's not much carbon monoxide coming off. In fact, in a man, a man gives off 0.4 cc per hour so you can imagine how much a rat gives off, and we are just measuring the radioactive carbon monoxide. During the first five hours the first point was essentially on the normal curve. The second day it was already above the baseline, so it's at least within 24 hours. Now, our last experiment indicates that it happens immediately within the first five hours.

DR. WEIBEL: Yes. I'm very happy about that.

COLONEL MUSGRAVE (USAF School of Aerospace Medicine): You seem to have a unique method here and a pretty good fix on the red cell life in these rats and I just wonder if you have, or plan, to look at the reverse situation under hypoxia to determine whether the characteristic response is entirely hematopoietic or whether you actually prolong the life span of the red cell using this technique.

DR. LEON: As a matter of fact, Dr. Landau is doing considerable work on this. He is in the Donner Laboratories at Berkeley, he and Dr. Winchell are doing considerable work on this. They are also doing considerable work using this technique on stressed red blood cells, that is, cells produced in reaction to hemorrhage, cells that are produced real quickly in response to some stress, and they show, in this case, that the mean potential life span is progressively shortened, depending on the severity of the stress or the amount of hemorrhage that is induced. They are doing work on hypoxia but in that case they do not find an increase in the mean potential life span. However, in splenectomized rats, they find an increase of four days in the mean potential life span, so the spleen is a selective organ and it has a selectivity of about four days. They do find an increased mean potential life span in hyp'd animals, but in this case you don't know if it is because of their decreased metabolism or what, and they are working on that right now.

DR. BENJAMIN: Dr. Leon, your findings have some significant effect on space flight insofar as at present astronauts are exposed to some 800 mm of mercury pure oxygen during the launch for several hours. This would indicate that there would be significant changes of red blood cells which we really did not account for so far. Do you have any plans to determine the effect in men?

DR. LEON: Well, in the first place, they also had nitrogen in the atmosphere.

DR. BENJAMIN: No, they do not, not during the launch. You see the man is in the suit, in a pure oxygen atmosphere at about 800 mm of mercury. The cabin of the spacecraft itself is a mixed gas atmosphere, 60% nitrogen, 40% oxygen.

DR. LEON: How long are they in this environment?

DR. BENJAMIN: Something like three or four hours.

DR. LEON: Well, Craig Fisher has invited us down next month to talk about doing some work, and I don't know what the plans will be, certainly, it's worth keeping what you said in mind. I assumed that they had been in a mixed gas environment.

DR. COULSTON: Let's get one thing straight, will you? You're working with a rat. A rat produces extramedullary hematopoiesis all over the place. I would suggest very strongly that some histopathology be done and that you pick a species that is more like man and not extrapolate too much from the rats to men. I think the experiment is beautiful. It's a nice experiment. But put a monkey in a little capsule. He doesn't have extramedullary erythropoiesis all over the place. This may explain your young

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population of cells very easily, because the mobilization of these young cells can be very quick--under the terms of all the torrs that you used and the oxygen you used. I don't know if this is correct but it could be.

DR. LEON: I agree with you, we should do it on another species. We hope we can do it on man.

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SESSION II

EVALUATION OF PROPELLANT TOXICITY

Chairman

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## THERAPY OF INTOXICATION BY AEROZINE-50

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### INTRODUCTION

Aerozine-50 is a missile fuel consisting of an equal mixture, by weight, of hydrazine and unsymmetrical dimethylhydrazine (UDMH). UDMH has a vapor pressure approximately 10 times that of hydrazine (156.8 vs. 14.4 mm Hg at 25 C) (Sutton, 1963). Because of this difference, an individual inhaling the vapors emanating from Aerozine-50 will suffer from predominately UDMH intoxication. On the other hand, if Aerozine-50 were spilled on skin, the UDMH would vaporize leaving a residue of hydrazine to be absorbed percutaneously, resulting in predominately hydrazine poisoning. Analytical chemical determinations have shown that the vapors emanating from Aerozine-50 are approximately 85 per cent UDMH and animal experiments indicate that approximately 95 per cent of the effect following skin contamination with Aerozine-50 is due to the hydrazine component of the mixture (Little, 1960).

On the basis of animal experiments, the outstanding effect following exposure to UDMH is central nervous system stimulation ranging from tremors to convulsions (Back, 1962). Serious systemic sequelae after exposure to hydrazine include: tremors, convulsions, fatty liver, interstitial nephritis, anemia, and disorders of glucose metabolism (Krop, 1954). Both compounds are irritating to the eyes, skin and respiratory tract (Sutton, 1963).

Pyridoxine, a form of vitamin B<sub>6</sub>, has been shown to be an effective protective and therapeutic agent in experimental UDMH toxicity (Back, 1963; Reeves, 1963; Weir, 1964). It has been found to be ineffective in the protection and treatment of acute experimental hydrazine poisoning (O'Brien, 1964; Tirunarayanan, 1956; Weir, 1964).

With this difference in the effectiveness of pyridoxine, the question arises as to what therapeutic regimen would be appropriate in the treatment of acute Aerozine-50 toxicity. Should pyridoxine alone be used, or should pyridoxine in addition to barbituates or other anticonvulsants be used? These experiments were designed to answer these questions.

#### MATERIALS AND METHODS

All experiments were performed using anhydrous hydrazine (97+%) and anhydrous UDMH (99+%) obtained from Matheson, Coleman, and Bell, Cincinnati, Ohio; pyridoxine hydrochloride (PIN-HCL) obtained from Eastman Organic Chemicals, Rochester, New York, and sodium phenobarbital from Merck & Co., Inc., Rahway, New Jersey.

In order to simulate the various routes of possible exposure, three types of hydrazine/UDMH mixtures on a weight basis were prepared: 80% UDMH/20% hydrazine (80U20H), simulating inhalation exposure; 50% UDMH/50% hydrazine (50U50H), simulating ingestion; and 80% hydrazine/20% UDMH (80H20U), simulating skin exposure.

White, male, non-fasting ICR mice weighing  $26 \pm 3$  (1 S.D.) grams were injected intraperitoneally (i.p.) with a dose of the mixture sufficient to kill 84 per cent of the mice in one week. Subsequently, pyridoxine HCl was injected intravenously in doses ranging from 1 to 320 mg/kg.

Preliminary investigation revealed that convulsions and death occurred as early as 10 minutes after receiving the 50 per cent and 80 per cent hydrazine mixtures. Therefore, a 5-minute interval to the injection of the therapeutic agent was chosen for these particular mixtures. A 30-minute interval was found to be satisfactory for the 80 per cent UDMH mixture. When Na-phenobarbital was used, it was administered i.p. 5 minutes after the hydrazine mixture.

A control group was injected i.p. with the toxic fuel mixture followed by injection of sterile H<sub>2</sub>O using comparable time intervals. Twenty mice were used at each dose level of therapeutic agent under study (10 treated and 10 control). The animals were housed in individual observation cages for four hours and during this time were observed for convulsions and death. Following this period, they were gang caged and mortality recorded every 24 hours for 7 days. All solutions were prepared daily using deionized distilled H<sub>2</sub>O and were administered in a volume of .01 ml/gram body weight.

The livers of the 10 mice surviving the 80 per cent hydrazine mixture and 10 normal livers were submitted as unknowns to two pathologists who compared them as to the amount of fat present on hematoxylin and eosin stain.

The statistical method of Litchfield and Wilcoxon (1949) was used in computing the dose effect curves and two tail students "t" test of proportions was used in evaluating the effectiveness of treatment.

## RESULTS

The seven-day i.p LD50, LD84 and the slope functions of hydrazine, UDMH and their mixtures are shown in table I. Figure 1 shows that hydrazine is the major contributor to the toxicity of the 50 per cent and 80 per cent hydrazine mixture. It also shows that the amount of UDMH in the LD50 of the 80 per cent UDMH mixture is greater than the amount in the LD50 of plain UDMH. This observation represents a statistically significant difference ( $p < .05$ ) and suggests that a low proportion of hydrazine may antagonize the toxicity of UDMH in hydrazine/UDMH mixtures. This finding is being investigated further at the Toxic Hazards Division, Wright-Patterson AFB.

TABLE I  
7-DAY LD50, LD84 AND SLOPE FUNCTION OF HYDRAZINE,  
UDMH AND THEIR MIXTURES

Compound	LD50 mg/kg	LD84 mg/kg	Slope Function
Hydrazine	82 ( 77- 87)*	92 ( 87- 98)	1.13 (1.07 - 1.20)
UDMH	125 (117-134)	150 (134-168)	1.21 (1.07 - 1.34)
50U50H	147 (140-154)	162 (156-168)	1.10 ( .95 - 1.14)
80U20H	190 (176-205)	230 (213-248)	1.20 (1.11 - 1.30)
80H20U	92 ( 84-101)	110 ( 99-122)	1.20 (1.08 - 1.33)

( ) \* numbers in parenthesis are 95% confidence limits

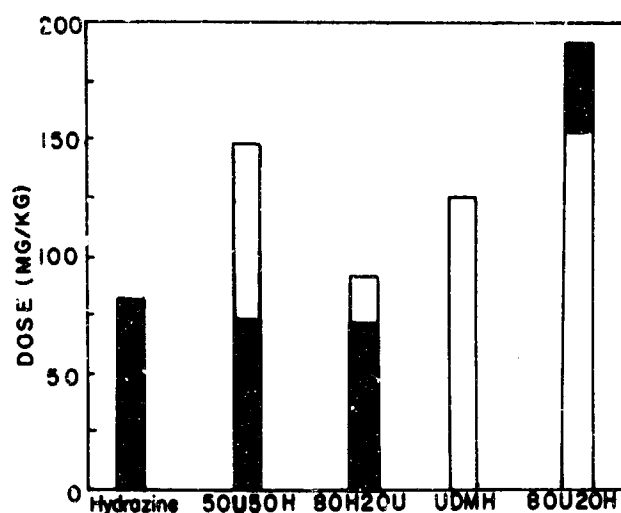


Figure 1. THE LD 50 OF HYDRAZINE, UDMH AND THEIR MIXTURES.  
The hydrazine component is shaded in.

The effectiveness of pyridoxine HCl in the treatment of toxicity due to these hydrazine mixtures is shown in table II. The 50 per cent UDMH mixture was sensitive to the PIN-HCL and 20 mg/kg i. v. resulted in 100 per cent survival. As the proportion of hydrazine was increased, the effectiveness of PIN-HCL decreased, requiring 80 mg/kg to achieve 100 per cent survival against the 50-50 mixture. These 100 per cent survivals are significantly ( $p < .001$ ) better than the untreated survival.

Even with doses of PIN-HCL as high as 320 mg/kg, 100 per cent survival could not be achieved against the 80 per cent hydrazine mixture. In fact, little improvement in survival occurred with doses of PIN-HCL above 80 mg/kg. Na-phenobarbital, therefore, was administered in addition to this dose of PIN-HCL.

The administration of 40 mg/kg Na-phenobarbital i.p. in addition to 80 mg/kg PIN-HCL resulted in 100 per cent survival of 10 mice poisoned with the 80 per cent hydrazine mixture (table III). This 100 per cent survival is better than the 70 per cent survival achieved with 40 mg/kg Na-phenobarbital alone (table III) or the 80 per cent survival obtained with 80 mg/kg PIN-HCL alone (table II). These results are highly suggestive even though the number of animals in this group was too small for meaningful statistical analysis. It is important to note that phenobarbital certainly did not increase the toxicity of the hydrazine mixture.



TABLE II  
PYRIDOXINE IN AEROZINE TOXICITY

Dose of Pyridoxine	% Mortality per 10 Mice		
	80U20H	50U50H	80H20U
Control*	82 (88)**	93 (75)	88 (74)
1 mg/kg	60 (50)		
2.5 mg/kg	30 ( 0)		
5 mg/kg	10 (10)		
10 mg/kg		50 (50)	
20 mg/kg	0 ( 0)	20 (10)	40 (40)
30 mg/kg		20 (20)	
40 mg/kg		20 (40)	50 (60)
60 mg/kg		10 (30)	
80 mg/kg		0 ( 0)	20 (30)
160 mg/kg			30 (40)
200 mg/kg			10 (20)
240 mg/kg			10 (10)
280 mg/kg			20 ( 0)
320 mg/kg			20 (10)

\* Based on 50 or more animals.

\*\* ( ) represents % convulsions during 4 hours' observation.

TABLE III  
PIN·HCL AND Na-PHENOBARBITAL IN 80H20U TOXICITY

Dose of Phenobarbital	Dose of PIN·HCL	Mortality per 10 Mice	
		Treated	Control
5 mg/kg		10 (8)*	8 (6)
20 mg/kg		6 (2)	10 (7)
40 mg/kg		3 (2)	10 (7)
20 mg/kg	80 mg/kg	1 (0)	8 (8)
40 mg/kg	80 mg/kg	0 (0)	10 (8)

\* ( ) represent convulsions per 10 mice.

Convulsions were usually followed by death (tables II and III). Occasionally an animal recovered. In table II, it is shown that 88 per cent of the untreated mice injected with the 80U20H mixture convulsed and 82 per cent of them died.

A very high percentage of all untreated animals died within 24 hours of the injection. In all treatment groups, death was delayed and seldom occurred after 72 hours (table IV).

The characteristic fatty liver of hydrazine poisoning was not seen in the 10 mice surviving the simulated skin exposure.

TABLE IV  
MORTALITY BY DAY\*

	1	2	3	4	5	6	7	Total
Treated ** 50U50H	$\frac{9}{60}$	$\frac{1}{60}$	$\frac{1}{60}$	$\frac{0}{60}$	$\frac{1}{60}$	$\frac{0}{60}$	$\frac{0}{60}$	$\frac{12}{60}$
Control	$\frac{56}{60}$	$\frac{0}{60}$	$\frac{0}{60}$	$\frac{0}{60}$	$\frac{0}{60}$	$\frac{0}{60}$	$\frac{0}{60}$	$\frac{56}{60}$
Treated ** 80U20H	$\frac{9}{50}$	$\frac{1}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{10}{50}$
Control	$\frac{41}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{41}{50}$
Treated 80H20U:								
PIN·HCL Alone	$\frac{15}{80}$	$\frac{0}{80}$	$\frac{2}{80}$	$\frac{3}{80}$	$\frac{0}{80}$	$\frac{0}{80}$	$\frac{0}{80}$	$\frac{20}{80}$
Phenobarbital Alone	$\frac{13}{30}$	$\frac{2}{30}$	$\frac{4}{30}$	$\frac{0}{30}$	$\frac{0}{30}$	$\frac{0}{30}$	$\frac{0}{30}$	$\frac{19}{30}$
PIN·HCL + Phenobarbital	$\frac{0}{20}$	$\frac{1}{20}$	$\frac{0}{20}$	$\frac{0}{20}$	$\frac{0}{20}$	$\frac{0}{20}$	$\frac{0}{20}$	$\frac{1}{20}$
Control	$\frac{109}{130}$	$\frac{0}{130}$	$\frac{6}{130}$	$\frac{1}{130}$	$\frac{0}{130}$	$\frac{0}{130}$	$\frac{0}{130}$	$\frac{116}{130}$

\* Numerator = number of dead mice; denominator = total number of mice injected.

\*\* Treated with varying doses of PIN·HCL alone.

## DISCUSSION

As mentioned earlier, pyridoxine has been shown to be effective against experimental UDMH toxicity. Our finding of its high degree of effectiveness in the treatment of simulated inhalation exposure was not surprising. Reported human cases of systemic hydrazine, UDMH and Aerozine-50 intoxication are rare. Frierson (1965) reported the case findings of six workers accidentally exposed to the inhalation of Aerozine-50. Their symptoms of nausea, vomiting, headache, tremors, shakiness and weakness cleared within 20 minutes following treatment with pyridoxine HCl. Two of the cases developed pulmonary edema which cleared with steroid therapy. These case reports lend additional support to our finding that PIN-HCL was effective against a simulated inhalation exposure.

Medina (1964) found that pyridoxine (0.5 mM/kg i.p.) did not afford protection to rats poisoned with an i.p. LD90 of 50% hydrazine/50% UDMH by volume. The reason for the difference between his results and ours is not known. It may perhaps be related to different methods of study - mice vs. rats; unbuffered vs. buffered solutions; i.v. vs. i.p. pyridoxine; and the matter of preparation of the hydrazine/UDMH mixtures. On a volume basis, the mixture will be relatively hydrazine rich as compared to our method of mixing the solutions on a weight basis.

Some support to our finding that PIN-HCL was effective against a simulated ingestion exposure to Aerozine-50 may be inferred from a case report by Reid (1965). He describes a sailor who accidentally ingested an unknown quantity of dilute hydrazine. A few minutes later he vomited and lapsed into unconsciousness. No convulsions were noted. Approximately 12 hours later the patient was critically ill with shallow and irregular respirations. At that time vitamin B<sub>6</sub> and 10 per cent dextrose were administered and the patient began to show hour by hour improvement. At the time of the case report he had partial return of his CNS function.

The exact mechanism of action of pyridoxine HCl in protecting animals from death due to UDMH is not known. It may be related to the findings that certain hydrazine derivatives inhibit the formation of pyridoxal-5-phosphate, an important coenzyme in the metabolism of gamma aminobutyric acid (GABA). GABA is felt to be one of the inhibitors of synaptic transmission in the central nervous system. This relationship, unfortunately, is not quite that simple and many conflicting reports exist in the literature as discussed by Holtz and Palm (1964).

Na-phenobarbital was chosen as the anticonvulsant because it has been shown to be a fairly effective protective agent against death due to plain hydrazine and UDMH (Weir, 1964). It is also a commonly used drug in the treatment of status epilepticus and is felt to produce less respiratory depression than shorter acting barbiturates (Gutrecht, 1969). Back et al (1963) have expressed concern that barbiturates may further depress respirations which have already been compromised by UDMH. Our finding that phenobarbital exhibited a fair degree of protection when administered alone is thus significant. With higher doses of the toxic mixture, this relationship may not exist. This is an area which warrants further investigation.

The finding that death seldom occurred after 72 hours and that the characteristic fatty liver of hydrazine poisoning was not seen in the mice surviving the 80 per cent hydrazine mixture may be related to the reports that the fatty livers of rats given sub-lethal doses of hydrazine return to normal in 72 hours (Amenta, 1962; Reinhardt, 1965). Cole et al (1953) also found that mice surviving hydrazine poisoning after treatment with a short acting barbiturate and Na-pyruvate did not show the characteristic fatty liver of hydrazine intoxication.

More research needs to be done with pyridoxine and phenobarbital against higher doses of the mixtures and preferably in a species when injection therapy can be instituted more readily after convulsions begin. Until more definitive work is done, however, the following approach to an individual who has suffered a severe skin exposure to Aerozine-50 seems reasonable. At the first sign of symptoms - nausea, emesis, or convulsions, pyridoxine HCl 25 mg/kg may be administered according to the method of Back et al (1963). In a 70 kg man this would be a total dose of 1750 mg. Four hundred milligrams of this dose may be injected i. v. and the remaining dosage given intramuscularly in several different sites. If the patient is convulsing, Na-phenobarbital (5 mg/kg) may be administered slowly by intravenous route, with careful monitoring of the patient's respirations and blood pressure. This dose of phenobarbital and pyridoxine may be repeated in 20 to 30 minutes if necessary.

Other aspects of therapy to be considered are: adequate hydration; removal of contaminated clothing; thorough washing of the skin; careful monitoring of the patient's vital signs, blood glucose, renal and liver function studies and hematologic status.

We wish to emphasize that this therapy is based on animal experiments but its use appears justified in an emergency situation in view of present knowledge.

#### SUMMARY

Pyridoxine hydrochloride was shown to be effective in the treatment of a simulated inhalation and ingestion exposure to Aerozine-50 in mice. It was only partially effective in the treatment of simulated skin exposure because of the predominance of hydrazine toxicity. Administration of phenobarbital in addition to pyridoxine resulted in 100 per cent survival of 10 animals simulating skin exposure. This study suggested a possible approach to therapy of Aerozine-50 toxicity.

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## DISCUSSION

DR. JACOBSON: I'd like to ask one question, and I'm not sure that I read your slide correctly, but in the treatment of the hydrazine--dimethylhydrazine mixture, with barbiturate and pyridoxine, you got essentially no mortality.

DR. AZAR (E. I. duPont de Nemours & Company): Yes.

DR. JACOBSON: But there is essentially no mortality among controls either. Did I read that correctly?

DR. AZAR: You read it incorrectly.

DR. THOMAS: Could we have the slide back?

DR. AZAR: Could I have the next to the last slide there, please?

(Slide)

DR. AZAR: I believe that the mortality usually was 8 out of 10 with the 80% hydrazine mixture, very few control groups where the mortality was less than 80%.

DR. THOMAS: By control, you mean untreated?

DR. AZAR: By control I mean untreated. Here I had ten out of ten die.

DR. JACOBSON: Yes, I read that as 10% die. That was my error.

CAPTAIN CHIKOS: I have two questions. One deals with the fact that you mentioned one of the compounds gives hypoglycemia, and I think from looking over some of Dr. Carter's data, it gets down to 30-40 milligrams percent. I wonder if you advocate any glucose therapy in that case.

DR. AZAR: I advocate careful following of the patient's blood glucose and if the patient is getting hypoglycemic, administer glucose. As a matter of fact--I don't know if you're familiar with the human experience with toxicity due to these compounds, but Reed reported a case of a sailor who was a little inebriated, who drank some dilute hydrazine on board ship, an unknown quantity, lapsed into unconsciousness, did not convulse, but vomited. About 12 hours later, on his flight from South Africa to Paris, he became critically ill, with shallow respirations and a decreased blood pressure.

At this time they started 10% glucose and Vitamin B6; they didn't specify the form, and the patient began to show hour by hour improvement. At the time of the case report he had had partial return of the central nervous system function. He still had some ataxia, residual, and his memory was intact and he was having a gradual return of the ability to write clearly.

CAPTAIN CHIKOS: The other comment I might make is that generally in dealing with convulsive disorders, in the clinical situation, you draw up about a 10 cc full syringe of one of the barbiturates. . . and that might be a better way of controlling convulsions, if they are occurring, rather than to give a predetermined dose and wait 20 minutes, because you can usually control the convulsions without causing respiratory depression. You just inject the barbiturate until you get cessation of convulsions and then stop at that point rather than a set dose.

DR. AZAR: You're right. I would agree with that. I meant to imply this, but I feel you should not exceed the dose of 5 milligrams per kilogram at a particular injection, and Dr. Back and his group have pointed out the need for caution in using barbiturates because these compounds may depress the central nervous system on their own, and so you must be careful in administering a barbiturate and monitoring respirations.

DR. LAWTON (Nav. Ord. Environmental Health Center): I was wondering why you chose phenobarbital rather than one of the faster acting barbiturates.

DR. AZAR: This is a commonly asked question and a point that is debated between neurologists. I'll clarify this by saying I am not a neurologist, but it is generally felt the longer acting barbiturates do not depress the respirations as much as shorter acting barbiturates. Another reason I chose phenobarbital in experimental hydrazine poisoning is that it has been shown to be an effective protective agent giving a fair degree of protection against experimental hydrazine poisoning. Does that answer your question?

DR. LAWTON: Yes. Normally, though, the people we're dealing with, say, aboard a ship where they might have an exposure, the first thing they would think about would be using amylbarbital.

DR. AZAR: I think this would depend on the physician seeing him, because, as I meant to imply, there are two schools of thought on this. Depending on which school of thought the physician was trained under. At Pittsburgh neurologists recommend for status epilepticus the use of sodium phenobarbital, intravenously. If the individual is using a shorter acting barbiturate he's going to have to be just as careful in watching the patient's respirations.

DR. HODGE: We tell our medical students that phenobarbital is almost unique among barbiturates for its anti-convulsant action.

DR. LAWTON: There's a difference in time, though, of onset. That's the problem. When we're dealing with a patient in an emergency situation, you want to get



him to stop convulsing as quickly as possible, and amylbarbital is much faster.

DR. AZAR: I would agree with you, but I have used sodium phenobarbital intravenously in the treatment of status epilepticus in an emergency situation and it has worked perfectly well for me.

DR. JACOBSON: A number of people seem rather interested in this. Do I interpret correctly that you all want to comment on barbiturate therapy in convulsions?

FROM THE FLOOR: Yes.

DR. JACOBSON: There's quite a bit of interest in this. Let me suggest, rather than taking the time away from the next paper, that we postpone this particular segment of discussion until the open forum after lunch.

DETERMINATION OF PROPOSED EMERGENCY EXPOSURE LIMITS  
FOR MONOMETHYLHYDRAZINE

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INTRODUCTION

Monomethylhydrazine (MMH) is a fuel used in rocket propulsion systems and consequently represents an exposure hazard to fuel manufacturing and handling personnel. The investigation of MMH toxicity has been a subject of great interest although the major research emphasis has been placed upon oral, intravenous, and percutaneous introduction of this chemical agent at lethal or supralethal dose levels. The comparative toxicities of hydrazine, UDMH, SDMH, and MMH were reported by Witkin (1956). Witkin demonstrated that there was little difference in toxicity between these compounds when administered by the oral, intravenous and intraperitoneal routes. The principal action of the hydrazines was effected upon the central nervous system producing severe convulsions resulting in death. Reynolds and Back (1966) reported decreased performance in trained primates at subconvulsant doses of MMH (2.5 mg/kg). The performance decrement appeared prior to or in the absence of clinical signs of MMH intoxication. Similar changes in the learned performance of cats have also been reported (Serman, 1968).

Acute inhalation studies of MMH toxicity have been reported (Jacobson, 1955; Haun, 1968) for four animal species (dog, monkey, rat and mouse). The pertinent findings of these studies conducted at near lethal atmospheric concentrations form the basis for the EEL studies.

A consistent finding in the nonfatal exposure of dogs to concentrations of MMH at or near the approximate  $LC_{50}$  value was a transient hemolytic anemia characterized by significant decreases in hematocrit, red blood cells, and hemoglobin which continued for several days postexposure. The destruction of red blood cells was accompanied by an increase in reticulocytes during the period of maximum decline of hematocrit levels. The process of recovery was complete within 30 days postexposure at which time normal values of the affected blood parameters were observed. Small groups of

Macaca mulatta monkeys were exposed to MMH to determine their response relative to the squirrel monkeys previously exposed. On the basis of results from one-hour exposure periods, and in view of the finding that other species have shown a rather predictable response to a CT (concentration x time) gradient, Rhesus monkeys are more resistant to the acute effects of MMH than either the squirrel monkey or the dog. One-hour exposures to concentrations at or above 170 ppm proved lethal, whereas exposures ranging from 120-160 ppm did not; this response places the Rhesus monkey between the rat and the mouse in susceptibility to MMH. An approximate one hour  $LC_{50}$  of 162 ppm MMH was determined for this species.

Exposure of Rhesus monkeys to maximal nonlethal concentrations of MMH has shown that the typical hematologic response exhibited by dogs is not demonstrated by this subhuman primate; there is but slight evidence of the anemia previously described for dogs. This finding is in agreement with reported studies on the effects of injected monomethylhydrazine (Pinkerton, 1967) which have shown that erythrocyte hemolysis in the monkey is minimal (approximately 10% reduction of RBC's with rapid recovery).

A common gross pathologic finding in all species, following lethal exposures to MMH, was pulmonary congestion with some hemorrhage, hepatic congestion of varying degree, and swelling of the renal tubular epithelium which was frequently glassine and eosinophilic in appearance. In large animals, whose brain tissues were examined, sub-arachnoid hemorrhage was frequently observed. This response was probably related to the severe convulsions observed, as was the consistent finding in dogs of remarkably bloodless spleens in which the sinusoids were virtually empty. In some cases, the splenic smooth muscle bundles appeared thickened and contracted.

The amount of visceral congestion and hemorrhage observed was not sufficient to produce death and could only be attributed to CNS damage as previously reported by Jacobson (1955).

In animals that survived near lethal exposures to MMH and were killed serially over a period of approximately 60 days postexposure, the visceral congestion was still apparent although not as severe as in those animals that died during exposure. The most common and persistent finding, however, was renal damage which ranged from mild swelling of the tubular epithelium to vacuolization and coagulative necrosis of those epithelial cells.

The primary purpose for the investigation and definition of MMH  $LC_{50}$  values was to form some basis for subsequent studies designed to assist in the interpretation of emergency exposure limits (EEL) for man. The basic philosophy pertaining to Short-Term Limits and Emergency Exposure Limits (EEL's) for inhalation of toxic substances has been frequently discussed by others (AIHA, 1964; NAS-NRC, 1964; Smyth, 1966) and will not be reiterated here. Very briefly, however, the term 'EEL' refers to a "single event" limited exposure which is not expected to incapacitate sufficiently to prevent escape. Such an exposure would be one where the occurrence was possible but unpredictable and where an individual so exposed would not encounter the substance again until careful study indicated either no injury or, in the event of injury, a complete recovery. Emergency inhalation exposures, therefore, are not to be associated with acceptable concentrations in a work environment atmosphere.

EEL's currently in use for MMH are 3, 7, and 10 ppm for 60, 30, and 10 minutes, respectively. On the basis of animal response to acute MMH exposures, and in view of the purpose for recommendation of EEL's, it appeared that higher values for MMH might have been warranted. Accordingly, some experimentation was necessary for clarification of this point, and the rationale for such experimentation is given below.

The plot of  $LC_{50}$ 's for each of four animal species tested is shown in figure 1. Plots of MMH concentration versus time permit extrapolation of the best fit lines through the respective  $LC_{50}$  values and yield a theoretical straight-line dose-response for each species. Thus, from a theoretical viewpoint, two rodent species (rats and mice) exposed continuously to 3.5-5 ppm of MMH would exhibit a 50% lethal response following approximately one week of exposure, whereas beagles and squirrel monkeys at this exposure would show 50% response at approximately one day (24 hours). Preliminary experiments with Rhesus monkeys, however, indicated a response midway between that shown for mice and for rats. The squirrel monkeys, therefore, represent the most susceptible species tested.

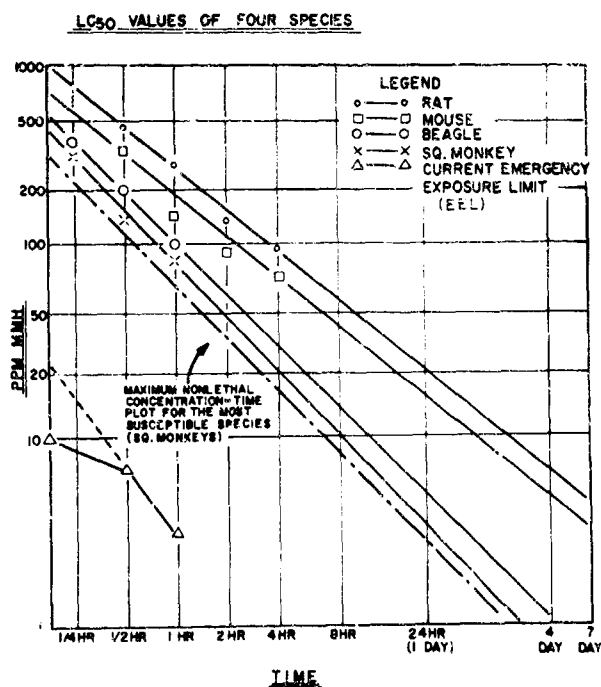


Figure 1. ACUTE TOXICITY OF MONOMETHYLHYDRAZINE (MMH)

It is well-known that the plot of theoretical values cannot be considered as a true dose-response relationship; the reactivity of MMH and the body defense mechanisms undoubtedly negate such an empirical response. Even so, the straight-line plots shown in figure 1 should represent the maximum possible response.

In this context, then, experiments were designed to indicate the validity of recommended EEL's for MMH. Current EEL's are shown as point plots in figure 1. Note that a straight-line dose-time relationship has not been recommended. If this had been applied, however, the 10-minute EEL would have been set at approximately 30 ppm of MMH rather than at the currently suggested 10 ppm.

The MMH concentrations selected for EEL testing on the four animal species (rat, mouse, beagle dog, and Rhesus monkey) were based on a CT dose of 900 ppm-minutes. This CT value was approximately 25% of maximum nonlethal concentrations for the most susceptible species, the squirrel monkey, and was also five times higher than the current EEL values adopted by the National Academy of Sciences - National Research Council Advisory Center on Toxicology. The selected concentrations were 15, 30, and 60 ppm MMH for single exposure periods of 60, 30, and 15 minutes respectively. Rodents and most dogs and monkeys were killed at 1, 3, and 7 days post-exposure for pathological evaluation. A second grouping of two dogs and two monkeys exposed to each MMH time-concentration group were used for pre- and postexposure blood sampling. They were also killed 30 days postexposure and tissue specimens were submitted for histopathology to determine reversibility of injury should any such have occurred from the MMH exposures.

Small groups of rodents, both rats and mice, were subjected to higher time-concentration exposures; namely, 150, 75, and 40 ppm MMH for 15, 30, and 60 minutes respectively.

## RESULTS

No significant differences between MMH exposed and control rodents were observed at any of the six selected EEL test concentrations. The mean pre- and postexposure weights of the MMH exposed rats and their controls are summarized in table I. The three individual groups of the 2250 ppm-minute and 900 ppm-minute CT exposures have been lumped together since there were no significant differences among them. Organ to body weight ratios are presented in table II for the same groups of animals. Again, no significant differences were observed at either the 900 ppm-minute or the 2250 ppm-minute dose levels.

No effects on body weight were observed in either dogs or monkeys exposed to the three 900 ppm-minute MMH exposure systems. At necropsy both species exhibited mild transitory changes which consisted of minimal congestion with slightly increased pigmentation of the renal cortex. These changes had completely resolved by the 30-day sacrifice period.

TABLE I

MEAN BODY WEIGHTS OF ALBINO RATS  
EXPOSED TO MONOMETHYLHYDRAZINE  
(weight in grams)

	Days Postexposure				No. of Rats
	0	1	3	7	
Exposed: CT = 2250	153	155	166	196	54
Controls	148	155	169	197	27
Exposed: CT = 900	163	167	180	203	54
Controls	163	170	182	205	27

TABLE II

MEAN ORGAN TO BODY WEIGHT RATIOS OF ALBINO RATS  
EXPOSED TO MONOMETHYLHYDRAZINE  
(organ weight/100 gram body weight)

	Heart	Lungs	Liver	Spleen	Kidney	No. of Rats
Exposed: CT = 2250	0.408	0.695	4.391	0.438	0.950	54
Controls	0.397	0.662	4.537	0.428	0.940	27
Exposed: CT = 900	0.426	0.667	4.638	0.416	0.938	54
Controls	0.406	0.656	4.583	0.408	0.924	27

Histopathologic evaluation of tissues of both dogs and monkeys necropsied at 1, 3, 7, and 28 days postexposure showed no significant differences from control animals. Mild pulmonary congestion was seen in some dogs on the third postexposure day but this is not believed to be related to the MMH exposure. It was not seen in animals necropsied at subsequent postexposure periods. An additional group of dogs exposed to 15 ppm MMH for one hour for evaluation of clinical chemistry parameters was also negative with respect to pathologic effects.

No clinical signs or symptoms of CNS changes could be observed in any of the four animal species exposed to MMH in the EEL test series. There was also no indication of respiratory irritation as had been observed during MMH inhalation exposures near the  $LC_{50}$  concentration.

Biochemical determinations on blood specimens taken from both dogs and monkeys exposed to the three concentration time periods remained within normal ranges, with the exception of glucose values. The drop in postexposure glucose values was believed to be related to increased laboratory temperature effects on the enzyme method used resulting from construction activities in the laboratory. Consequently, an additional series of five dogs each were exposed to MMH concentrations of 15 ppm and 8 ppm for a 60-minute period. Baseline values were collected on all dogs for 4 weeks prior to the MMH inhalation exposure. These MMH exposed dogs and their controls were tested twice weekly for 7 weeks postexposure. Composite weekly mean values for the blood glucose levels are listed in table III. There were no significant differences between the exposed dogs and their controls.

TABLE III  
BLOOD GLUCOSE LEVELS IN MMH  
EXPOSED DOGS AND THEIR CONTROLS

		Glucose mg/100 ml		
		Exposed CT = 900	Exposed CT = 480	Controls
Pre- Exposure	1	99	101	101
	2	101	101	107
	3	99	95	103
	4	107	107	112
Post- Exposure	1	106	106	110
	2	107	106	109
	3	103	104	106
	4	103	105	108
	5	98	99	105
	6	100	99	103
	7	98	104	102

## DISCUSSION

An important factor in considering establishment of inhalation exposure limits for MMH is its rapid oxidation in air and in the animal. Air oxidation begins immediately and is relatively complete within one hour as reported by Vernot (1967). The primary oxidation products of MMH in air are methane and nitrogen. Small amounts of other carbon containing compounds including  $\text{CO}_2$  are also formed. Dost (1966) has described the metabolic fate of MMH in rats using  $^{14}\text{C}$  labeled material injected intraperitoneally. The in vivo oxidation of MMH reaches a maximum rate within two hours and is essentially complete 3-4 hours postinjection. Approximately 30% of the metabolized MMH is excreted as methane and about 10% as  $\text{CO}_2$ . The bulk of the remaining carbon containing metabolite appears in the urine.

These findings on the metabolic fate of MMH with respect to rate of decomposition agree with the reported findings of Reynolds and Back (1966). The performance decrement induced in primates by MMH began 1-2 hours after injection and decreased significantly 4-5 hours after injection.

The induction of performance decrement in primates required an MMH dose of 2.5 mg/kg. Calculation of the maximum possible inhaled dose of MMH in the studies reported herein show it to be 0.5 mg/kg for the primate or 20% of the dose required for performance decrement.

In view of the negative findings in all species from MMH inhalation exposures of 900 ppm-minutes and in both rats and mice at 2250 ppm-minutes and the safety factor for performance decrement, we recommend an upward revision of the Emergency Exposure Limit values for monomethylhydrazine as shown below:

<u>Minutes</u>	<u>PPM MMH</u>
10	90
30	30
60	15



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## DISCUSSION

MR. WANDS: Dr. MacEwen was kind enough to let us see these data sometime ago. He came down to Washington and made a presentation to the Committee on Toxicology of this paper, and as a result of that the Committee has considered his recommendation. About three weeks ago they reached a conclusion, made a recommendation, and I would like to just throw that out right now for the information of the group. The committee has recommended to adopt your values of 90 ppm for 10 minutes, 30 ppm for 30 minutes, and 15 ppm for 60 minutes. They do have, however, one question, and they are holding this back as a tentative recommendation from their organization at the moment, until they can be assured that there is no problem of high irritancy from these concentrations. They are particularly concerned about such things as the possibility of bronchospasm in event of a spill, or something like that, which might be acutely incapacitating. You know we did check with you a few weeks ago on this question and your response at that time was that the stuff smells pretty much like ammonia and is certainly no more irritating than ammonia, and perhaps not as strongly; so with this tentative question in the back of their minds about irritancy, the committee has adopted your recommendation. I might add one other comment that here is one more example today of what the Committee on Toxicology can do once it is given some adequate data. Again, in the past, the data did not justify anything more than what they had recommended and particularly, they did not feel a CT relationship necessarily existed, particularly for the short terms, and this was why the ten-minute figure was lower than might have been extrapolated. I do thank you very much, Dr. MacEwen, for making these data available. It's a very nice piece of work.

DR. MAC EWEN: Thank you. I think this shows the problem of establishing EEL values. All that was available didn't really give them very good basis for the establishment of these, and that is what frequently happens, I'm afraid.

DR. JACOBSON: Are there other questions or comments? If there are none pressing right now, I'll ask them to be held until this afternoon, and what I would appreciate some discussion of, Ralph, is a little bit more of why the value for ten minutes is so much different, but I'm just warning you that if we have time I'd like to quiz you on that this afternoon.

## ACUTE TOXICOLOGY AND PROPOSED EMERGENCY EXPOSURE LIMITS OF NITROGEN TRIFLUORIDE

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### INTRODUCTION

The acute toxicity of nitrogen trifluoride ( $\text{NF}_3$ ) has been shown by previous investigators (Ruff, 1931; Torkelson et al, 1962; Dost, et al, 1968) to be due to massive formation of methemoglobin, with death ensuing from the resulting anoxia. These studies did not, however, quantitate the acute toxicity of  $\text{NF}_3$  by determination of  $\text{LC}_{50}$  values. The investigations reported here were, therefore, undertaken to evaluate the  $\text{LC}_{50}$  at 15, 30, and 60 minutes in rats, mice, beagle dogs, and rhesus monkeys. Additionally, experiments were conducted to determine what short-term exposure levels of  $\text{NF}_3$  would have no significant effects on all species tested. The latter series of tests was carried out to aid in setting up realistic emergency exposure limits. Present EEL values for  $\text{NF}_3$  proposed by the Committee on Toxicology, Advisory Center on Toxicology, National Academy of Science, National Research Council are 100 ppm for 30 minutes and 50 ppm for 60 minutes corresponding to a dose level of 3000 ppm-minutes.

### MATERIALS AND METHODS

Male Sprague Dawley strain rats and ICR mice were exposed in groups of 10 in a bell-jar chamber of 30 liter volume. Male and female dogs and monkeys were exposed in a Rochester Chamber described by Haun (1969). The gaseous  $\text{NF}_3$  was introduced into the chamber air stream after passage through a sodium fluoride trap to remove any hydrogen fluoride which might have been present in the  $\text{NF}_3$ .

A gas chromatographic procedure for the analysis of chamber concentrations of  $\text{NF}_3$  using a silica gel column was developed which gave 95% confidence limits of  $\pm 5\%$  relative. Analyses could be performed every two minutes, which was satisfactory in the monitoring of a 15-minute exposure. A spectrophotometric technique was used for the concurrent determination of blood turbidity, methemoglobin and oxyhemoglobin by

measurement of the absorbance of diluted blood at wavelengths of 720 m $\mu$ , 630 m $\mu$ , and 578 m $\mu$ . Although this technique is not as precise as that of Evelyn and Malloy (1938), it is not subject to turbidity errors as may be the case with that procedure.

For the determination of LC<sub>50</sub> values, a total of 150 rats, 150 mice, 34 dogs, and 32 monkeys were used. LC<sub>50</sub> calculations were made by a computerized version of the method of Litchfield and Wilcoxon (1949). For each concentration tested during EEL experiments, 6 dogs, 6 monkeys, 30 mice and 30 rats were used, with identical numbers of control animals.

Monkeys and dogs surviving 60-minute LC<sub>50</sub> exposures were held for six weeks during which time hemoglobin, red blood cells, hematocrit, reticulocytes, turbidity and the presence of Heinz Bodies were determined at intervals of three to five days. Gross pathological examinations were made on animals dying of acute exposure and on the survivors at necropsy at varying intervals after exposure. Tissues were also taken for histopathological examination.

Animals exposed to the EEL test concentrations of NF<sub>3</sub> were held for four weeks postexposure. The same parameters were measured as in the animals surviving the 60-minute LC<sub>50</sub> exposure.

## RESULTS AND DISCUSSION

### Acute Exposures

The LC<sub>50</sub> values calculated from the acute exposure data are listed in table I. Examination of table I illustrates the fact that the toxicity of NF<sub>3</sub> is not very high. Even at one hour, almost one percent by volume is necessary to cause deaths in 50% of the animals. The other interesting point revealed by table I is that there is a great difference in the response of different species to NF<sub>3</sub>. At the 15-minute exposure interval, twice as high a concentration is necessary to cause 50% deaths in dogs as in mice.

The lines in figure 1 are "best fits" to the data shown in table I, plotting NF<sub>3</sub> LC<sub>50</sub> values versus the reciprocal of the exposure time. If the classical C-T relationship were applicable, one would expect a straight-line plot through the origin. In the case of rats and dogs, the expected relationship is demonstrated. However, the lines for monkeys and mice do not pass through the origin but show an intercept. Another factor of interest in the figure is the inversion of toxicity of NF<sub>3</sub> to rats and mice shown at about 40 minutes exposure. Below this exposure interval, the material appears to be more toxic to mice than rats while above it the extrapolated line indicates that rats are more subject to the lethal effects than mice. In order to test the validity of extrapolation and to determine whether there really was an inversion of toxicity at longer exposure times, rats and mice were exposed for 120 minutes to 4500 ppm of NF<sub>3</sub>. This concentration is about midway between the theoretical LC<sub>50</sub> values of the two species as read from figure 1. The mice experienced 20% mortality and the rats 90% from this exposure indicating that the plots in figure 1 may be validly extrapolated.

TABLE I

LC<sub>50</sub> VALUES FOR VARIOUS SPECIES, PPM

<u>TIME, MIN.</u>	<u>RATS</u>	<u>MICE</u>	<u>DOGS</u>	<u>MONKEYS</u>
15	26,700	19,300	38,800	24,000
30	11,700	12,300	20,200	14,100
60	6,700	7,500	9,600	9,200

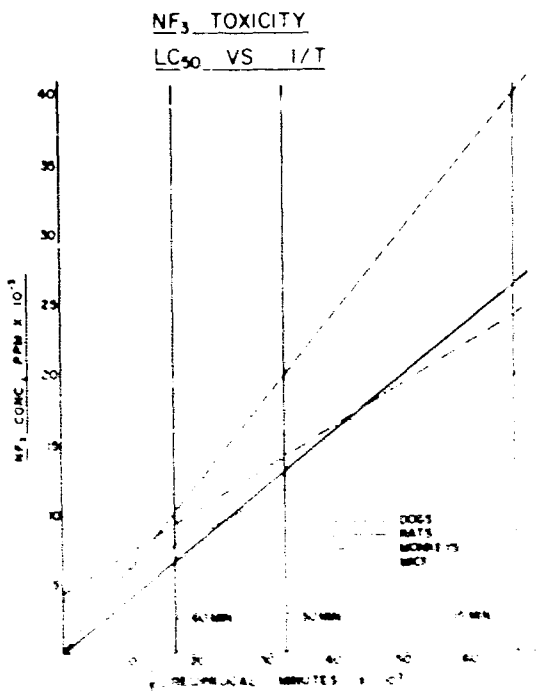


Figure 1

The equations corresponding to the plots in figure 1 are shown in table II and again demonstrate that rats and dogs show a simple C-T relationship while monkeys and mice do not. There is, however, a linear dose-effect relationship for all species.

TABLE II

LC<sub>50</sub> - TIME EQUATIONSLC<sub>50</sub> IN PPMTIME IN MINUTES

RATS	$LC_{50} \times T = 390,000$
DOGS	$LC_{50} \times T = 600,000$
MONKEYS	$LC_{50} = 4,300 + \frac{300,000}{T}$
MICE	$LC_{50} = 3,800 + \frac{240,000}{T}$

Survival in large animals acutely exposed to NF<sub>3</sub> was found to be dependent upon the amount of methemoglobin produced or, conversely, on the amount of oxyhemoglobin remaining in the blood as measured immediately after exposure. Conversion of more than 75% of the oxyhemoglobin to methemoglobin, leaving a residue of less than 4 grams percent oxyhemoglobin, invariably resulted in death of the animal. Hourly measurement of methemoglobin levels starting immediately after exposure showed a rapid linear decrease with a corresponding increase in oxyhemoglobin until preexposure levels of methemoglobin were attained some 5-7 hours postexposure. Figure 2 is a diagram of the course of methemoglobin conversion to oxyhemoglobin which was seen in all dogs surviving acute exposures.

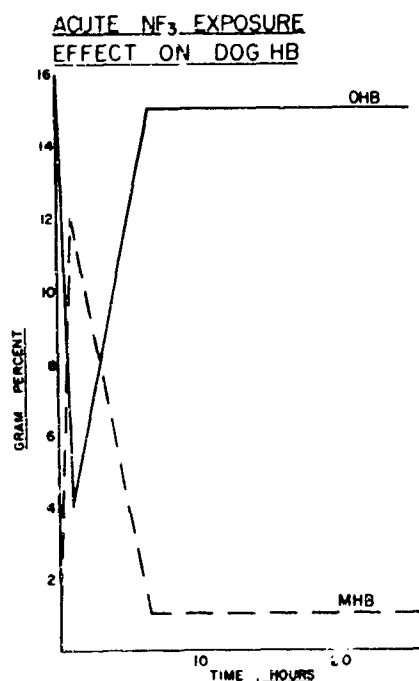


Figure 2

Although methemoglobin returned to baseline levels, oxyhemoglobin concentrations after disappearance of methemoglobin were about one gram percent lower than pre-exposure values. Concurrently with this, the turbidity of the diluted blood was higher after exposure. Since the production of Heinz Bodies is a common result of methemoglobin formation by such materials as phenylhydrazine (Jandl et al, 1960), the possibility existed that the postexposure turbidity was caused by denaturation of the hemoglobin and subsequent Heinz Body formation. Staining the blood sampled after exposure with cresyl blue, a standard vital stain for Heinz Body detection, did reveal the presence of numerous Heinz Bodies in the red cells. Hemolyzed blood used for spectrophotometric analysis was centrifuged, and staining the residue with cresyl blue disclosed that it consisted mainly of quasicrystalline granules identical to those seen previously in the red cells. This was strong evidence that the turbidity in the blood of exposed animals was, in fact, due to the presence of Heinz Bodies.

Symptomatology during acute exposures appeared to result from anoxia and included emesis and cyanosis due to methemoglobin. Gross and histopathological findings in those animals that died during or after exposure were consistent with a diagnosis of anoxia and massive methemoglobin formation. These findings included lung congestion, edema, and focal hemorrhage. Additionally, all organs of the viscera were congested, showing a pale to dark brown color depending on the degree of methemoglobin formation. In all animals that survived acute exposures and held two weeks or longer, no gross or histopathological effects of exposure were noted.

In order to follow more precisely the course of hematological changes caused by exposure to  $\text{NF}_3$ , a group of dogs was exposed to 9600 ppm, the  $\text{LC}_{50}$  concentration for 60 minutes, and the fourteen survivors held for 40 days. Figures 3 and 4 depict the hematological changes with time resulting from acute exposure to  $\text{NF}_3$ . The close correspondence between turbidity and Heinz Bodies is readily seen in figure 3. Differences between these two plots may be explained by the fact that the Heinz Body measurement consisted of counting all red blood cells containing Heinz Bodies. Multiple inclusion bodies in individual cells, although seen, were not recorded. Heinz Bodies attained a maximum some two days after exposure, remained fairly constant at a plateau for about two weeks, and decreased to zero after three weeks. Reticulocyte determinations gave fairly scattered results, but appeared to peak at about 6% after three weeks, returning to normal after 30 days.

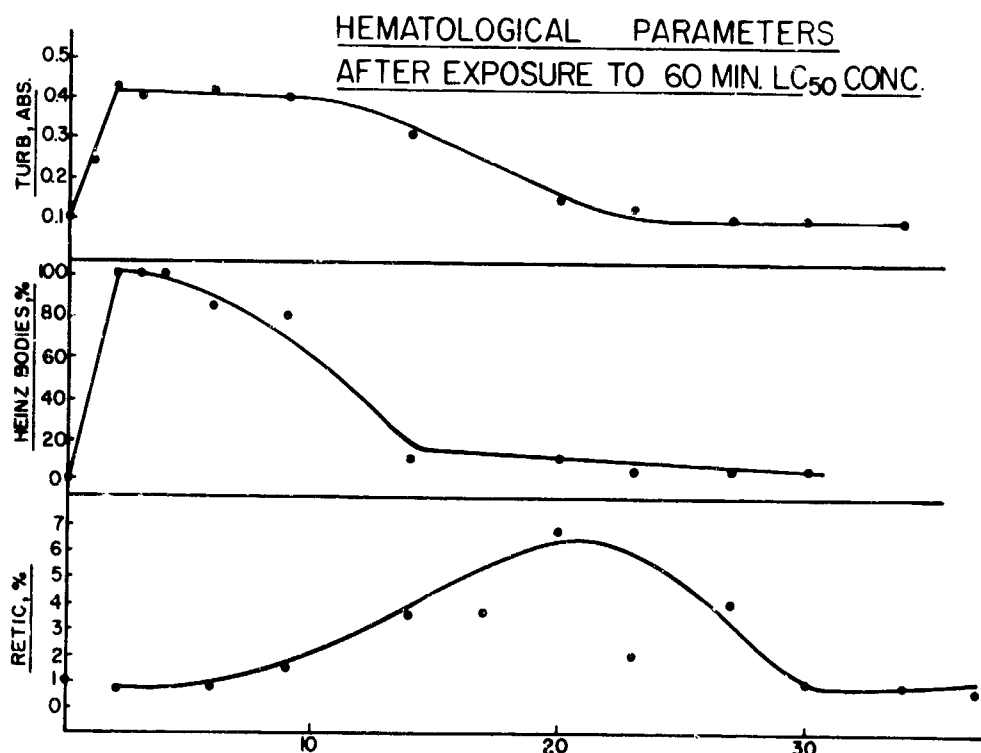


Figure 3



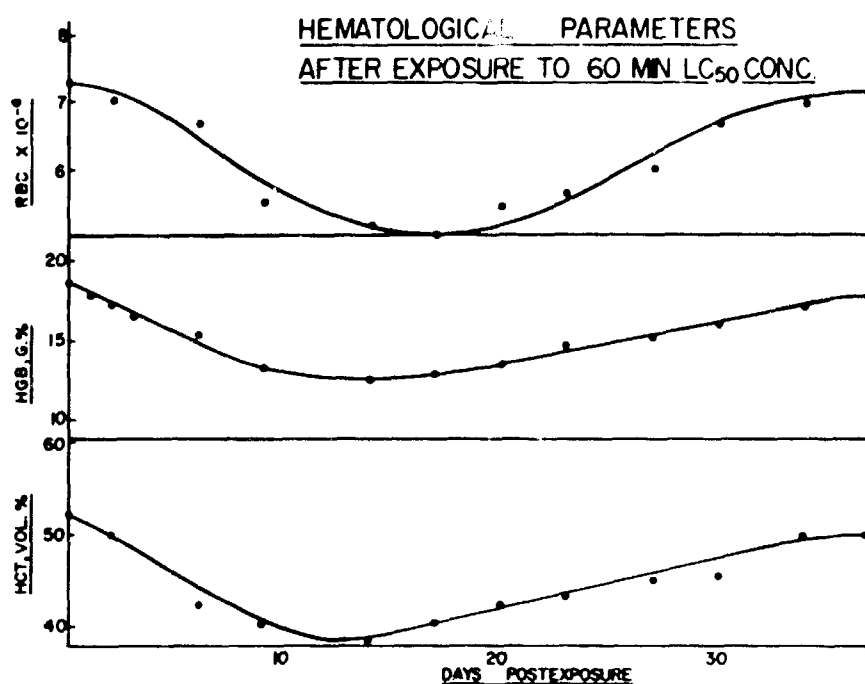


Figure 4

As illustrated in figure 4, red blood cells, hemoglobin and hematocrit all showed approximately the same 33% decrease to a minimum about 2 weeks postexposure. The hematologic measurements then increased slowly, reattaining normal values at the end of the holding period. The picture presented is one of a typical Heinz Body anemia caused by intoxication with chemicals such as aniline, nitrobenzene and phenylhydrazine (Neches and Allen, 1969), (von Oettingen et al, 1947) and Selwyn, 1955).

#### Emergency Exposure Limit Studies

As noted previously, the NAS-NRC has set a tentative NF<sub>3</sub> EEL dose of 3000 ppm-minutes for 30 and 60 minutes. Since our work had indicated that the acute effects of NF<sub>3</sub> were caused by anoxia due to methemoglobinemia and that the anemia following acute exposure was reversible, it was decided to perform a number of experiments at subacute levels to determine whether the NAS-NRC EEL dose represented a realistic value or whether it could be revised upward.

The acute effects of carbon monoxide, like those of NF<sub>3</sub>, result from anoxia and, since the EEL dose of CO is based upon a concentration converting 15% of the oxyhemoglobin to carboxyhemoglobin, an NF<sub>3</sub> dose converting 15% hemoglobin to methemoglobin was thought to represent an EEL value suitable for testing. Initial experiments indicated

that a 120,000 ppm-minute dose of  $\text{NF}_3$  would cause 15% conversion of hemoglobin to methemoglobin. Therefore, dogs, monkeys, rats, and mice were exposed to this dose for 15, 30, and 60 minutes. As table III shows, the aim of 15% conversion was fairly well achieved in all the exposures. No adverse symptomatology was noted during exposure, and no effects on body weight gain during the 28-day postexposure holding period or on organ to body weight ratios at necropsy were seen. Gross and histopathological examination revealed no differences from unexposed animals.

TABLE III

METHEMOGLOBIN PRODUCED BY  $\text{NF}_3$ ,  
APPROXIMATE 120,000 PPM-MIN. DOSE

<u>TIME, MIN.</u>	<u>CONC. PPM</u>	<u>MHB, % RELATIVE</u>	
		<u>DOGS</u>	<u>MONKEYS</u>
15	7,000	19.2	16.5
30	3,500	12.1	13.1
60	2,000	14.4	10.3

Hematological parameters of the dogs and monkeys were followed during the 28 days postexposure with no significant change from controls on the part of monkeys. The dogs, however, demonstrated decreases in HCT, HGB, and RBC with minimum values being reached at time periods comparable to those after acute exposure. As is shown in table IV, the average decrease in these parameters was 16% compared with 33% for the acute studies. Thus, even though the tested dose was only 20% of the  $\text{LC}_{50}$  dose, the decreases in HCT, HGB, and RBC averaged 50% of the  $\text{LC}_{50}$  values. These effects were important enough to remove from consideration the use of 120,000 ppm-minute  $\text{NF}_3$  as an EEL dose because individuals with various types of anemia might be adversely affected by such decreases.

TABLE IV

COMPARATIVE HEMATOLOGICAL EFFECTS IN DOGS  
EXPOSED TO TWO LEVELS OF NF<sub>3</sub>

<u>PARAMETER</u>	<u>LC<sub>50</sub> DOSE</u> <u>(600,000 PPM-MIN.)</u>		<u>EEL DOSE</u> <u>(120,000 PPM-MIN.)</u>	
	<u>DAYS TO -</u> <u>MINIMUM</u>	<u>% DECREASE</u>	<u>DAYS TO -</u> <u>MINIMUM</u>	<u>% DECREASE</u>
HCT	9-17	35	7-10	15
HGB	9-14	33	10	18
RBC	14-20	30	10-14	16

Since it appeared that methemoglobin formation was causing the Heinz Body anemia, a study was instituted to determine the maximum NF<sub>3</sub> dose producing no measurable methemoglobin in dogs within the precision of the technique used. Dogs were exposed to various concentrations for 60 minutes, immediately after which the methemoglobin content of the blood was measured. Reference to table V demonstrates that an approximate 30,000 ppm-minute dose showed 2.2% conversion of hemoglobin to methemoglobin. Since this is below the precision of the technique, it was concluded that this NF<sub>3</sub> concentration had no effect on the blood.

The EEL experiments previously carried out using a 120,000 ppm-minute dose were repeated for all species, using the 30,000 ppm-minute dose. Again, as expected, no changes in body weight gain rate were noted during the 28-day postexposure period or in organ to body weight ratio at necropsy. Gross and histopathology were also negative. This time, in dogs, the hematological parameters HCT, HGB and RBC showed no decrease when compared to controls during the postexposure holding period. All the measurements made indicated that exposure to 30,000 ppm-minutes at 15, 30, and 60 minutes had no detectable effect on any of the species tested.

TABLE V

METHEMOGLOBIN PRODUCED IN DOGS  
BY VARIOUS DOSES OF NF<sub>3</sub> \*

<u>CONC., PPM</u>	<u>DOSE, PPM - MIN.</u>	<u>MHB, % RELATIVE</u>
1,075	65,000	9.7
510	31,000	2.2
290	17,000	0.0

\* 60 MIN. EXPOSURE TIME

The results of the final EEL exposures provide strong evidence that the proposed NAS-NRC EEL dose of 3000 ppm-minutes is unrealistically low. That it was set low is understandable in view of the fact that very little data were previously available on the effects of NF<sub>3</sub> at subacute concentrations. However, the studies reported in this paper have demonstrated the safety of subacute exposure sufficiently to recommend revising the NAS-NRC EEL dose upward to 30,000 ppm-minutes. Table VI compares the recommended NF<sub>3</sub> EEL concentrations and times with the current proposed NAS-NRC values.

TABLE VI

COMPARISON OF RECOMMENDED  
EEL'S WITH NAS-NRC VALUES

<u>RECOMMENDED</u>		<u>NAS-NRC</u>	
DOSE-30,000 PPM-MIN.		DOSE-3,000 PPM-MIN.	
<u>TIME, MIN.</u>	<u>CONC., PPM</u>	<u>TIME, MIN.</u>	<u>CONC., PPM</u>
15	2,000	—	—
30	1,000	30	100
60	500	60	50

SUMMARY

The  $LC_{50}$  concentrations of  $NF_3$  at 15, 30, and 60 minutes have been determined for mice, rats, dogs, and monkeys. We confirmed that the immediate effects of acute exposure are caused by extensive methemoglobin formation and resulting anoxia. Animals surviving acute exposures experience a Heinz Body anemia with RBC, HGB, and HCT decreasing 33% to a minimum by the end of the second week postexposure. Recovery of hematological values to preexposure levels is attained in 40 days. In dogs, sufficient anemia is caused by a dose level of 120,000 ppm-minutes to invalidate that dose as an emergency exposure limit. At 30,000 ppm-minutes, however, no detectable anemia occurs, and no other toxic effects are discernible. The results of experiments conducted at subacute levels justify recommending an upward revision of the EEL dose to 30,000 ppm-minutes from the proposed NAS-NRC value of 3000 ppm-minutes.

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## TECHNIQUE OF RADIORESPIROMETRY

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### INTRODUCTION

In metabolic studies with mammals, conventional techniques for the measurement of respiratory activities do not provide information on the metabolic fate of a specific carbonaceous compound in the test animal. However, the advent of radiotracer methodology has made it possible to trace metabolic conversion of  $^{14}\text{C}$ -labeled carbon atom(s) of a given compound to respiratory  $^{14}\text{CO}_2$ . Measurement on the rate and extent of formation of respiratory  $^{14}\text{CO}_2$  in tracer experiments offers valuable information on metabolic pathway(s) handling the test compound in normal and stressed animals. This technique, generally referred to as radiorespirometry (Wang, 1967), has been widely used in studies with microorganisms and plants, in addition to mammals.

On the basis of the objective of the experiment and the nature of the test compound, radiorespirometric experiments with mammals can be classified into two basic types. The first type is concerned with the study of the metabolism of compounds known to occur in the test animal. Under this type are the studies of metabolism of carbohydrates, fatty acids, amino acids and other biological compounds. The second type is concerned with compounds foreign to the test animals. These include carbonaceous pharmaceuticals, toxicants and other compounds that do not occur naturally in animal systems. Needless to say, design concept of a radiorespirometric experiment is specific to the type of the experiment.

### DESIGN OF EXPERIMENT

In experiments with mammals, proper design of a radiorespirometric experiment is essential to obtain meaningful findings. Design parameters include route of administration of  $^{14}\text{C}$ -labeled compounds; chemical and radiochemical levels of labeled compounds administered; labeling position of the test compound; and duration of experiment (Wang, 1964).

Labeled compounds are administered to the test animal generally in two different fashions. In the so-called "single dose experiment",  $^{14}\text{C}$ -labeled compound is administered to the test animal all at once by means of intravenous injection, intraperitoneal injection or stomach-tube feeding. Findings in this type of experiment, while useful, are more or less qualitative in nature, since the administered compound, if it can be utilized, is not metabolized under a physiological steady state. In other words, body concentration of the labeled compounds is initially high and declines as metabolism proceeds. It will be difficult to draw inferences from this type of radiorespirometric data on such issues as metabolic rate and biological turnover time.

To gain more meaningful information on the kinetics underlying the metabolism of the test compound, continuous intravenous infusion is the chosen method for the administration of the labeled compound. With rats, infusion of the labeled compounds is readily accomplished by way of an indwelling cannula surgically placed in the anterior vena cava, usually through the right jugular vein (Weeks and Davis, 1964). The rate of infusion ranges from 0.5 to 0.9 ml per hour with rats weighing about 250 grams. With primates, infusion can be carried out by way of either the intravenous route and in the case of squirrel monkeys, subcutaneous injection is at present the preferred route. By the use of the continuous infusion technique, one is able to adjust the rate of compound administration to correspond to the rate of metabolic turnover, thereby simulating a metabolic steady state in rat with respect to the test compound. Under these conditions, the radiorespirometric data can be readily analyzed to yield quantitative information pertaining to the metabolism of the test compound. Moreover, such data can be used to estimate relative participation of concurrent pathways for the metabolism of such compounds as glucose (Wang, Prince, Dost, and Johnson, in press).

The chemical level of labeled compounds to be administered to the test animals is naturally dependent on the type of experiment. If the test compound occurs naturally in animals, consideration should be given to the biological concentration as well as the metabolic rate of the compound. Administration of an excessive amount of labeled compound will overload the metabolic mechanism and may induce abnormal anabolic activities or extensive renal excretion. On the other hand, tracer quantity (usually less than a milligram) of labeled compounds, once administered, may be easily overwhelmed by the relatively large quantity already present in the metabolic pool, giving rise to less meaningful radiorespirometric data. If a test compound does not occur naturally in animals, the level to be administered should generally be low with due consideration given to the toxicity or other physiological effect that may be inflicted by the compound.

In order to ascertain the proper level of the labeled compound to be used in radiorespirometric experiment, it is often desirable to conduct a series of experiments at various chemical levels. Examination of the experimental findings including yield of respiratory  $^{14}\text{CO}_2$ , amount of labeled compound incorporated into the animal constituents, and amount of labeled compound excreted will provide one with guidelines to choose a proper level of the labeled compound. In experiments involving the continuous infusion technique, consideration should be given to the dynamic metabolic capability of the animal in handling a specific compound. It is essential that the labeled compound be administered at the rate corresponding to the metabolic capability to yield radiorespirometric data pertaining to metabolism under physiological conditions.



The radiochemical level of labeled compound is dependent on the nature of the test compound, the route of administration, the type of experiment and the sensitivity of the radioactivity measuring device. With an ion chamber-electrometer system, radioactivity of the test compound is generally below a few microcuries in radiorespirometric experiments with rats.

The position of the labeling in the test compound is chosen on the basis of the objectives of the experiment. Compounds labeled with  $^{14}\text{C}$  uniformly or at a readily oxidizable carbon atom (such as a carboxyl group) can be used in radiorespirometric experiments to provide one with general information on the metabolic fate of the compound. However, if one desires to establish the pathways for the metabolism of a test compound containing several carbon atoms, it will be desirable to use a series of  $^{14}\text{C}$ -specifically labeled compounds individually in concurrent radiorespirometric experiments. Analysis of the rates and yields of respiratory  $^{14}\text{CO}_2$  production, derived from individually-labeled carbon atoms of the compound, often unveil the nature of the metabolic steps as well as the metabolic intermediates derived from the test compound.

With a single dose experiment, the duration of experiments should be such that all metabolic events with respect to the labeled compound can be observed. In other words, experiments should be allowed to proceed until the production of respiratory  $^{14}\text{CO}_2$  declines to an insignificant level. This is of particular importance if one desires to compare radiorespirometric data from a series of concurrent experiments using the test compound labeled with  $^{14}\text{C}$  at various specific positions. When the continuous infusion technique is used for the administration of the labeled compound, the experiment should be allowed to proceed until the rate of formation of respiratory  $^{14}\text{CO}_2$  reaches a plateau. At that point, it can be generally assumed that the radiorespirometric data are those reflecting utilization of the test compound at a metabolic steady state. In addition, in order to gain information on the depletion of the labeled compound, infusion should then be stopped and the experiment allowed to proceed until the formation of respiratory  $^{14}\text{CO}_2$  reaches an insignificant level. Such a procedure would permit one to establish an inventory of the administered labeled compound with respect to  $^{14}\text{CO}_2$  production, biological incorporation and excretion.

### RADIORESPIROMETER

The experimental setup to apply radiorespirometric experiments to small animals (Wang, 1967; Dost, Reed and Wang, 1964) is shown in figure 1. In essence, the respiratory  $\text{CO}_2$  produced by the animal in a respiration chamber, generally 1.5 to 2 liter in size, is swept with a stream of air at a rate of 500 ml/hr through an ion chamber 1,000 ml in size. The ionization current generated by the  $^{14}\text{CO}_2$  is collected and measured by means of a vibrating reed electrometer. The resulting amplified current is then channeled into a digital voltmeter to provide data readout in digital form. A data-scanning device is utilized to collect the digital data sequentially from concurrent radiorespirometer systems and recorded by a fast printer. These data can also be transferred onto punched tape to be processed by a computer. The respiration chamber for experiments with rats is depicted in figure 2 (Wang, Prince, Dost, and Johnson,

in press) showing also the arrangement for continuous infusion of the labeled compound. With animals such as squirrel monkeys, the respiration chamber can be replaced by a glass dome covering the head of the monkey, with the flow rate of air through the dome maintained at 1,000 ml/min. With monkeys held on a chair, the administration of labeled compounds can be readily accomplished by intravenous injection or intra-peritoneal injection in single dose experiments or subcutaneous infusion in continuous infusion experiments.

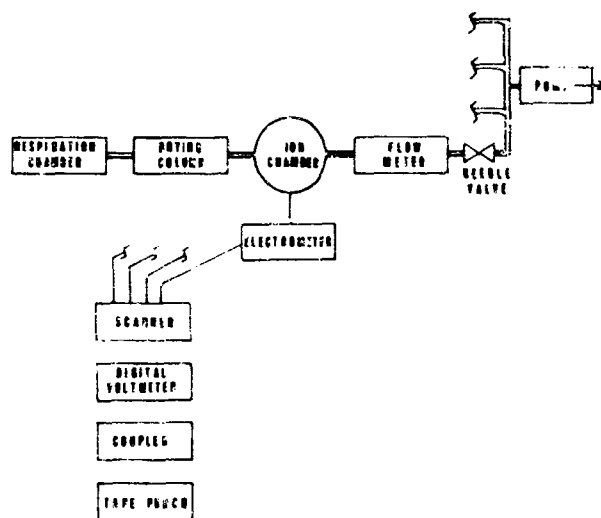


Figure 1. ANIMAL RESPIROMETRY AND DATA ACQUISITION SYSTEM FOR FOUR SIMULTANEOUS EXPERIMENTS

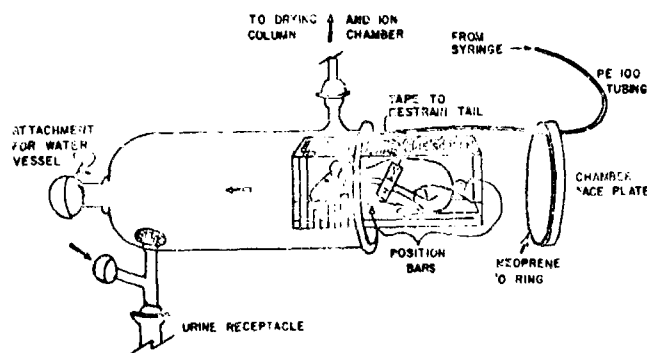


Figure 2. METABOLISM CHAMBER AND RESTRAINT CAGE FOR CONTINUOUS INFUSION OF LABELED SUBSTRATES. (Chamber support, face plate clamp, and water supply not shown).

## APPLICATIONS

The usefulness in applying radiorespirometry to elucidate metabolic pathways of biological compounds can best be illustrated by a study on the utilization of  $^{14}\text{C}$ -labeled glucose by rats. Previous work done in this regard (Wang, Snipper, Bilen, and Hawthorne, 1962) involved single dose experiments and revealed qualitative information that glucose is metabolized by way of the glycolytic, the pentose phosphate and the glucuronic acid pathways. The data did not permit an estimation on the relative participation of the respective pathways. More recently, in order to understand better glucose metabolism in rats, the continuous infusion technique has been applied in several series of radiorespirometric experiments (Wang, Prince, Dost, and Johnson, in press).

The first series of experiments was designed to ascertain the proper level of glucose to be administered via intravenous infusion to rats. As shown in figure 3 when glucose-3 (4)- $^{14}\text{C}$  was administered to the rats at levels below 500 mg/hr; i. e., at levels of 50, 150, and 340 mg/hr, a metabolic steady state with respect to the labeled glucose was realized. When the rate of infusion was 500 mg/hr, the rate of  $^{14}\text{CO}_2$  production reached a constant level only in the last two hours of the infusion period. Moreover, even after termination of glucose infusion at 10 hours, respiratory  $^{14}\text{CO}_2$  was produced at a significant rate over a period of the next 5 hours. The cumulative yield of  $^{14}\text{CO}_2$  at the end of the experiment was found to be 67%, somewhat higher than that observed in experiments with infusion rate of 50 mg/hr. Evidently, with such a high rate of substrate administration, the rat was not capable of metabolizing promptly the infused glucose resulting in the channeling of a significant portion of the administered glucose into depletable anabolic reserves. It is noted that when the rate of glucose infusion was 50 mg/hr, only 57% of the radioactivity of the glucose was converted to respiratory  $\text{CO}_2$ . Evidently, a sizable portion of the administered glucose-3 (4)- $^{14}\text{C}$  was trapped in various endogenous pools by way of exchange reactions associated with anabolic processes, resulting in a limited conversion of C-3 and C-4 of glucose to respiratory  $\text{CO}_2$ .

Analysis of the findings in this series of experiments led one to believe that glucose should be infused continuously to rats at the rate of 150 mg/hr. At this level, the administered glucose can be promptly routed into catabolic processes without too much interaction with endogenous reserve.

In the second series of experiments, various  $^{14}\text{C}$ -specifically labeled glucose samples were administered individually to the rat at the rate of 150 mg/hr. Infusion was continued for 10 hours and the experiment was allowed to proceed for another 6 hours. The findings in this series of experiments are given in figure 4. Considerable information on glucose metabolism can be obtained by an analysis of the radiorespirometric data observed under metabolic steady-state conditions. Thus, the rate of  $^{14}\text{CO}_2$  production from various carbon atoms of glucose was found to follow the order  $\text{C-3} > \text{C-4} \gg \text{C-2} > \text{C-1} > \text{C-6}$ . On the basis of these data, it has been estimated that in rats, glucose is metabolized 83% via the Embden-Meyerhof-Parnas glycolytic pathway; 11% via the pentose phosphate pathway and 6% via the glucuronic acid pathway (Wang, Prince, Dost, and Johnson, in press). This information constitutes the baseline understanding of normal metabolism in rats and is hence of primary importance to the study of the effect of external stresses upon glucose metabolism in rats.

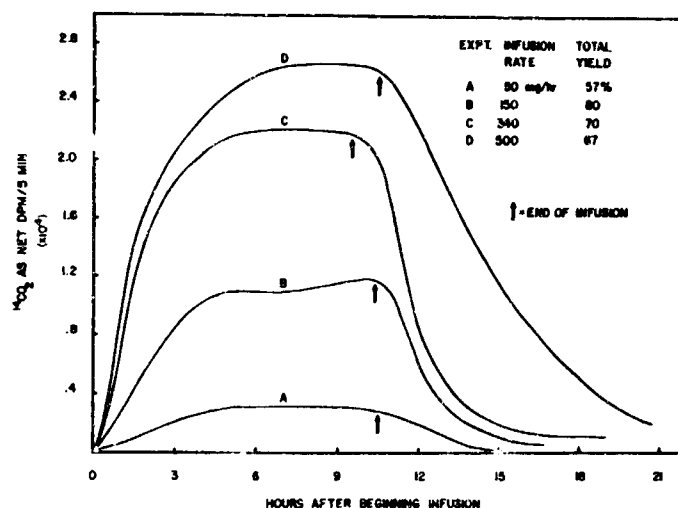


Figure 3. RATES OF  $^{14}\text{CO}_2$  PRODUCTION FROM RATS UTILIZING GLUCOSE-3(4)- $^{14}\text{C}$  ADMINISTERED BY CONTINUOUS INTRAVENOUS INFUSION.

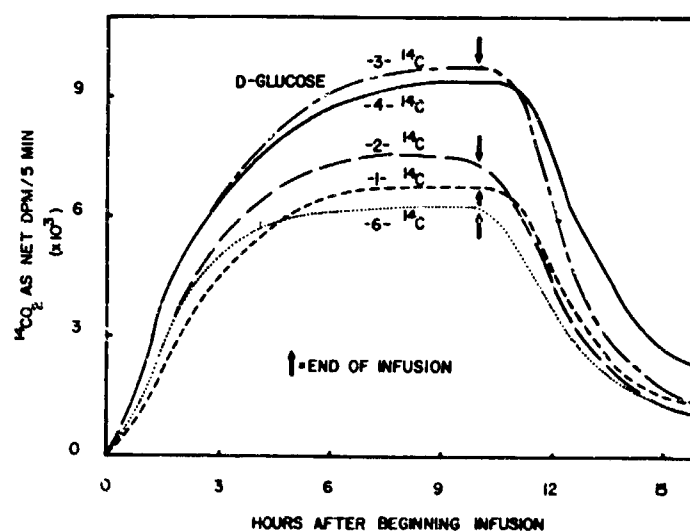


Figure 4. RATES OF  $^{14}\text{CO}_2$  PRODUCTION FROM RATS UTILIZING  $^{14}\text{C}$ -SPECIFICALLY LABELED GLUCOSE SUBSTRATES ADMINISTERED BY CONTINUOUS INTRAVENOUS INFUSION.

The application of radiorespirometry in studies of metabolism of non-natural occurring compounds in rats can be illustrated by experiments with  $^{14}\text{C}$ -labeled MMH (Dost, Reed, and Wang, 1964). The findings obtained in a series of experiments in which the labeled MMH was introduced into the rat intraperitoneally at 0.12, 0.24, and 0.48 mm/kg of body weight are graphically given in figure 5. Results obtained in these single-dose experiments revealed that: (a) MMH can be readily converted to respiratory  $\text{CO}_2$  and  $^{14}\text{CH}_4$ ; and (b) the metabolic capability of rats in handling MMH is limited. Thus, when MMH was administered in higher doses, proportionately greater amounts were found to be retained in tissue.

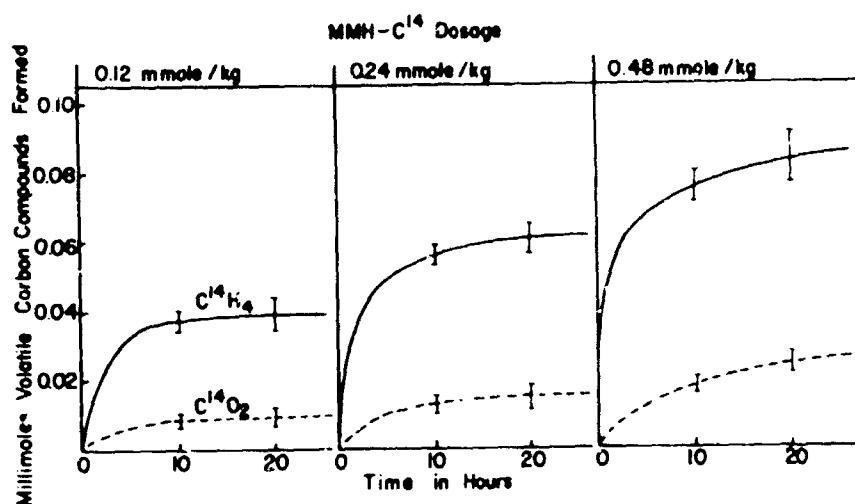


Figure 5. CUMULATIVE PRODUCTION OF  $^{14}\text{CO}_2$  AND  $\text{C}^{14}\text{H}_4$  BY RATS ADMINISTERED MONOMETHYLHYDRAZINE- $^{14}\text{C}$ .

Radiorespirometric experiments in toxicological and physiological studies can be illustrated by a series of experiments in which the effect of pure oxygen atmosphere upon glucose catabolism in rats was studied and the findings compared with the radiorespirometric pattern for glucose utilization by normal rats (figure 4). With glucose-3(4)- $^{14}\text{C}$  as test compound, it is noted that the conversion of these two carbon atoms to respiratory  $\text{CO}_2$  (figure 6), is much more rapid in the initial phase and reached a metabolic steady state considerably sooner than that observed with rats breathing in air. However, the steady rates of conversion of these two carbon atoms to respiratory  $\text{CO}_2$  remains the same regardless of the nature of the atmosphere. The findings can be interpreted on the basis that during the initial phase, the administered glucose is diluted to a less extent by the endogenous glucose.

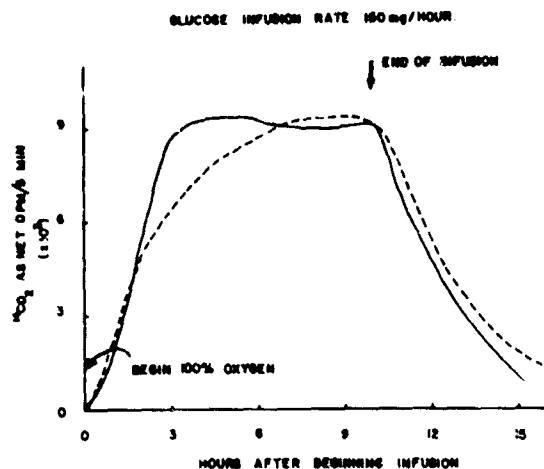


Figure 6. CONVERSION OF CONTINUOUSLY INFUSED GLUCOSE -3, -4- $^{14}\text{C}$  TO  $^{14}\text{CCO}_2$  BY RATS IN AIR AND PURE OXYGEN.

The rate and extent of conversion of other glucose carbon atoms to respiratory  $\text{CO}_2$  in rats breathing in oxygen are found to be the same as that observed with rats breathing in air except in the case of C-6 of glucose. In the latter case, as shown in figure 7, at the metabolic steady state, C-6 of glucose was converted to  $\text{CO}_2$  significantly greater in extent under oxygen atmosphere. These results lead one to conclude that among three concurrent glucose pathways functioning in rats, the glucuronic acid pathway is preferentially stimulated when rats are breathing in pure oxygen atmosphere.

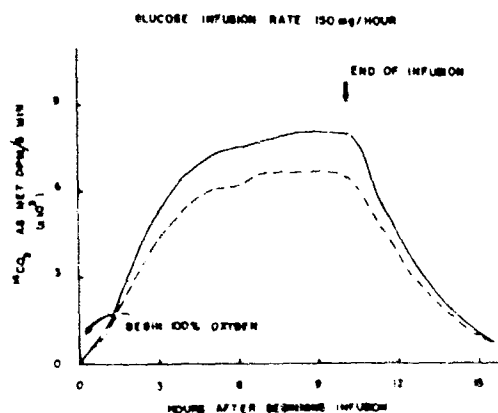


Figure 7. CONVERSION OF CONTINUOUSLY INFUSED GLUCOSE-6- $^{14}\text{C}$  TO  $^{14}\text{CO}_2$  BY RATS IN AIR AND PURE OXYGEN.

In figure 8 are shown the results obtained in an experiment in which a rat was infused continuously with glucose-6- $^{14}\text{C}$  until a metabolic steady state was reached, at which time the atmosphere in the respiration chamber was abruptly changed from air to pure oxygen. It is interesting to note that the rate of  $^{14}\text{CO}_2$  production began to rise almost immediately to a higher level and was maintained at the elevated plateau until infusion was terminated. Evidently, glucose catabolism in rats via the glucuronic acid pathway is rather sensitive to the partial pressure of oxygen in the environment.

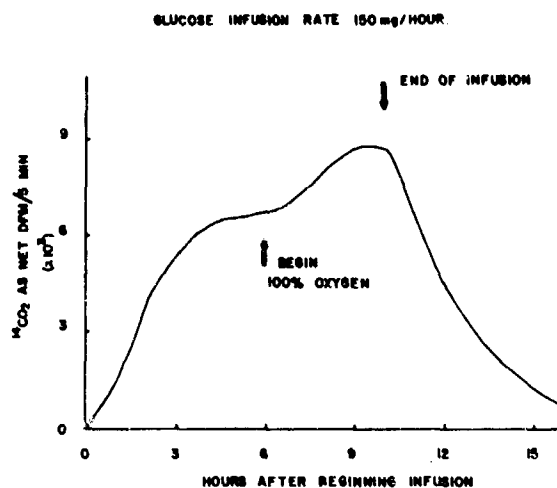


Figure 8. CONVERSION OF CONTINUOUSLY INFUSED GLUCOSE-6- $^{14}\text{C}$  TO  $^{14}\text{CO}_2$  BY RATS IN AIR AND PURE OXYGEN.

Radiorespirometry offers toxicologists a useful technique in studying the metabolic fate of toxicants and the biochemical mechanism underlying intoxication. The technique permits one to gain insight on the dynamic events functioning in normal and intoxicated animals.

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## DISCUSSION

DR. TOWNSEND (University of Texas Medical School): Professor Wang, you gave some figures, I know you were moving rather rapidly and I didn't get the figures on the utilization of glucose, the percentage of utilization in the different pathways. Would you repeat that please?

DR. WANG (Oregon State University): Yes, in the case of rats, the glucose catabolizes 83 percent by the way of the glycolysis, 11 percent pentose phosphate, 6 percent by glucuronic acid. Lately we have done some work with squirrel monkeys, by means of continuous infusion. It looks like a quite different picture from the rats. This may give us some excitement. Insofar as we can see, glycolysis is not as important in squirrel monkeys as it is in rats, and the pentose phosphate pathway gets a bit more important. As a general reference to human beings, we don't know the exact figures; nobody has used continuous feeding. We do know that pentose phosphate occurs probably somewhere between 10 and 30 percent and glucuronic pathway is a trace. I think some effort will be made to test human subjects on glucuronic acid pathways.

DR. JACOBSON: I would like to raise one question for Mr. Vernot. You commented that the emergency exposure limits for carbon monoxide were based on the conversion of hemoglobin at 15 percent, and you suggested that the conversion of the hemoglobin, up to 15 percent methemoglobin by nitrogen trifluoride would be analogous. Was this because you thought the one was equivalent to the other in the impact on the health of the animal or merely as a device in studying the material further?

MR. VERNOT: My feeling is that at 15 percent I would assume the impact would be quite comparable whether you were removing oxyhemoglobin by forming carboxy-hemoglobin or by forming methemoglobin.

QUESTION: Yet you actually proposed emergency exposure limits at levels that would produce appreciably less than 15 percent methemoglobin?

ANSWER (Mr. Vernot): That's true and that followed because when we analyzed the postexposure hematology of the animals that had been exposed to concentrations which would form 15 percent methemoglobin, the amount of decrease, the percentage of decrease in the hematological parameters, red blood count, hemoglobin and hematocrits, was serious enough for us to consider that concentration to be invalid, or to be questioned to the extent that it would not be acceptable as an EEL.

MR. WANDS: One thing I think we must keep in mind whenever we are dealing with any methemoglobin forming agent is that there is a genetic variation in humans in

their ability to reduce methemoglobin. I don't know the extent of this genetic factor within the normal population. I don't think anybody does. There are some fairly inbred groups such as the Eskimos and possibly some of the Indian tribes where it is a fairly common finding, but the total incidence within the population we don't know, and we must be a little careful about this.

MR. VERNOT: That is true.

DR. DOST (Oregon State University): We found that, for one thing, after exposure to  $\text{NF}_3$  of either rats or dogs, a residual level persists that we had initially considered to be methemoglobin, and which after discussing the problem with Dr. Hodge, realized was probably sulfhemoglobin; a level of perhaps 3 to 5 percent persists up to about two weeks and quite probably longer. In all likelihood, since this is a relatively irreversible change it is probably related to the turnover-time of the red cells. Another thing, we find with dogs maintained in an  $\text{NF}_3$  atmosphere in a closed circuit, rebreathing type system, that the animal will come to equilibrium in terms of methemoglobin formation and reduction. At  $\text{NF}_3$  concentrations of anywhere below 2500 ppm, at least in the short range, the only problem is with irritation and so forth. Now obviously there is an upper limit as to how long this can be carried forward. Our conclusion is that the inhalation lethality is due simply to methemoglobin formation. When the  $\text{NF}_3$  is injected intraperitoneally, the result is a rather quick rise in methemoglobin which is usually almost completely reduced by 80 or 90 minutes, and then at about two hours after the injection the animal expires. We have been strongly tempted to go looking at other heme proteins, particularly cytochromes, to see what sort of oxidation has taken place in these systems. Obviously the distribution of this very insoluble gas is going to be different when administered intraperitoneally. There is some possibility of systemic distribution with the i.p. route where there almost certainly is not upon inhalation. If it doesn't go in the red cells during transit across the pulmonary capillaries, it probably is not going to get in at all.

MR. VERNOT: I think I must agree with the latter two of your statements, but the first one is about sulfhemoglobin. My feeling is that there is no sulfhemoglobin produced. At least, I could never see any evidence of it spectrophotometrically. I think that what appears to be sulfhemoglobin is the turbidity that results from the Heinz Body formation. If you use the Evelyn-Malloy technique for methemoglobin measurements, you don't subtract the turbidity out completely, and so it is read as what the literature calls sulfhemoglobin, but I just don't believe it is there.

QUESTION: How soon do you see the Heinz Body formation?

ANSWER: Immediately afterwards.

QUESTION: Immediately afterwards?--This could very well be then.

COLONEL BELL: Mr. Wands, have you been requested to re-evaluate your EEL for  $\text{NF}_3$  as you were in the case of MMH, and if so, what are these values; if not, what do you feel the committee's action will be in dealing with orders of magnitude difference?

MR. WANDS: First of all, Colonel Bell, this is the first opportunity we have had to receive a request like this. These people have just recently completed this study and they have not yet presented a formal request to the committee for review. We expect to get a copy of the manuscript for distribution to the committee and will probably be studying it in the next few months. At this point I would hesitate even to try to predict the reaction of the committee. There is only one member of it present here today. Mr. Rowe is here; the rest of the committee, the entire committee was invited and we are very grateful to Dr. Thomas for this, most of them however, right now are on their way to Tokyo; so that they preferred to go there rather than Dayton.

DR. THOMAS: I can't blame them for that, can you?

MR. WANDS: This is, as you point out, an order of magnitude different; however, I would call attention to the fact that the original EEL's were a best guess on the basis of some very very meager data. Once again we are seeing some solid data to take a look at after the committee has had a chance to study this. I think that we might well treat this presentation and the requested suggestion today as a formal request to the committee. I don't think it will require any special paperwork from the Air Force or any of the other sponsoring agencies to trigger action by the committee. They frequently have in the past taken action on modifications of EEL's on their own initiative as soon as data became available. This would be the case here.

MR. ROWE (Dow Chemical Company): Just to amplify what Ralph has said, I would like to make a plea to people who are developing data such as we have heard here today, which I think is excellent, but I would like to have them at least get a little feel for what these concentrations do to humans. To set an EEL or something like this, that a person can't walk into without being almost immediately incapacitated by virtue of being unable to breathe it, or else eye irritation, is a little bit foolish. I think I'd like to suggest perhaps, Colonel Bell, that you see if you can't get these people to get a little better human data to give us some threshold limits as far as subjective or objective signs are concerned.

MR. VERNOT: We have already made tentative plans for human exposures and we are just waiting for O.K.s and the word "go".

DR. JACOBSON: Are there other questions or comments? Well, then, I think maybe this would be a suitable time for Mr. Wands to expound on the subject that I have already raised that is actually relevant to the nitrogen trifluoride paper, so I raised it in connection with the monomethylhydrazine paper and that was:--when the response data of animals (and presumably man), plotted concentrations against time result in a straight line, why then do the recommended limits for man not follow a straight line, but have an appreciably lower value for the ten minute period? And I had asked Mr. Wands to expound on that.

MR. WANDS: I cannot tell you in terms of molecular biology why. I can tell you somewhat in terms of the thinking of the Committee on Toxicology and some of the basic principles involved in establishing EEL's.

Several years ago when this concept of EEL's was first developed, there were two separate committees considering the question. One of them for the American Industrial Hygiene Association, and Dr. Jacobson was a member of that committee at the time, and they wrote a position paper or a philosophy document. At the same time the Committee on Toxicology of the National Research Council was considering the question and they too published a philosophical document. When the two came out independently, they were almost identical; at least the phraseology said the same thing, although they used slightly different words. One of the things that was included in these concepts is that the emergency exposure limit will tolerate a certain degree of response, adverse effects upon the exposed individual in view of the fact that it is an emergency situation, and in view of the fact that these are intended for military people. However, they also stipulate that this adverse response, immediate adverse response, or delayed, shall not incapacitate the individual from carrying out the necessary action in the emergency. This means that there has been somebody that has kicked over a carboy of some material on the lab floor, maybe the only emergency action is to leave the room. On the other hand, if it has been a broken valve or something like that requiring somebody to go in and take some positive action, like shutting off a control line or something of that nature, or dragging some other individual out, these emergency actions must not be interfered with by the anticipated exposure.

I think that there is another feature here that has to be considered also--the purpose of the EEL's. We do not intend that anyone should ever be exposed to any of these emergency exposure limits. These are not like a threshold limit value where one anticipates that somebody is going to work at these levels, but are primarily for planning purposes in order that you may establish appropriate exclusion radii, appropriate limits on the quantities of material in any one container that might be subject to rupture, or something of that nature. So these are guidelines to avoid exposures rather than guidelines for concentrations to which one can be exposed.

Now with regard to the linearity of the values, we felt that in choosing the times of exposures for which the committee makes these recommendations, namely ten minutes, thirty minutes and sixty minutes, we felt that any exposure of an individual beyond sixty minutes is hardly still in the category of an emergency exposure to that individual. By this time there has been plenty of opportunity for some sort of corrective action to be taken, some sort of protective gear to be applied or whatever. If the emergency continues beyond that, things are in pretty rough shape, and this is not the sort of thing that is being contemplated.

At the other end of the line, the ten minute figure was selected as being about the only thing for which one could realistically expect to get some data. One does not get good breathing zone atmospheric concentrations in anything much less than this. Perhaps you can go down to five minutes on occasion, but certainly one cannot say that this particular volume of air which is inspired in this portion of the respiratory cycle has a particular measurable concentration of contaminant in it. This is a

difficult thing to say, particularly in an emergency where you have not generated equilibrium concentrations throughout the entire space; so that ten minutes was the minimum time the committee felt was a realistic period to set data for.

The dose relationships utilized for these things at the 30 and 60 minutes are primarily, and I hope Mr. Rowe will correct me if I overstep my bounds or misinterpret the committee's comments, but the 30 or 60 minute doses are primarily based upon systemic effects--what might be anticipated in a human from such an exposure. The ten minute figure includes not only the anticipated systemic effect, but also includes these subjective features that V.K. just spoke about such as physical interference with an individual's ability to carry out an emergency reaction. Such things as excessive eye irritation to the point where you can't see because you are crying so hard, such things as bronchospasm as I mentioned this morning, some of these immediately effective irritant responses, certainly represent a nonlinear subjective response by the individual and therefore they could not be expected to follow a CT relationship, and yet they are quite critical. So that the ten minute figure is looked at in a little bit different light than the 30 and 60 minute figures. I think that as you go through all of the EEL's which the Committee has recommended you will find a fair degree of linearity with the 30 and 60 minute figures because after all the CT concept does more or less apply over a time span of that sort, but when you get down to the ten minute figure as with the MMH figures that Dr. MacEwen had on the screen this morning, the original ten minute figure was cut back because of irritant potential. Even now the Committee is still not too sure that the new ten minute figure should be a linear thing. They are hoping that there will be some human data on the irritant action of this material at short term exposures.

DR. JACOBSON: Am I correct that an additional reason for the low values at ten minutes is the assumption of a higher minute volume operating in those circumstances?

MR. WANDS: In establishing all of these, the Committee has made some attempt to estimate the minute volumes which might be accomplished, and yes, the ten minute anticipated minute volume in an emergency situation is much greater than for an hour. Yes, this is another feature.

DR. JACOBSON: Ralph, you had commented that these two groups had studied the problem, the philosophy of EEL's, and independently had come up with similar recommendations. This was not so independent. There were overlapping memberships and the position paper of one group was made available to the other group in drawing up its position papers.

MR. WANDS: Thank you. That was prior to my history of association with the Committee.

DR. JACOBSON: I think the only significant disagreement between the two groups was the lower time limits, that had any importance.

MAJOR THEODORE: I just wanted to comment that phenobarbital, just as Dr. Azar pointed out earlier today, has been one of the drugs of choice for anticonvulsive activities and for a number of reasons that were brought up earlier today. Also it is one of the few barbiturates that doesn't interfere with oxidative metabolism in the brain. I think it is the only one. I think now that neurologists have found a better anticonvulsant, however, and Valium is probably going to be the drug of choice in the future. It has been used in Europe for a number of years, but it just cleared the Federal Drug Administration in the last several years. So I would say that current with today's thinking, intravenous Valium is probably the best drug that I am aware of as far as treating acute convulsive disorders.

DR. AZAR: My comment on this is that I think that Valium certainly is a drug that should be looked into as a possible therapeutic agent. We did not do this so I cannot say whether it would work or not. I would like to comment that I believe and I don't know if I am pronouncing his name properly or not but J. A. Gutrecht in Kahn's Current Therapy, 1969 edition, recommends sodium phenobarbital five milligrams per kilogram intravenously as a treatment of choice in status epilepticus. I suspect, as you do, that Valium may become the drug of choice.

MAJOR THEODORE: I concur with what you say. Short acting barbiturates have been very popular among the pediatricians in the past. It may be that little monkeys are like little people, I don't know, but certainly many of the neurologists prefer phenobarbital because once you suppress the convulsive disorder, and intravenously it acts quite rapidly, you also want to keep the abnormal focus of the brain suppressed. There is no real benefit in suppressing an irritable focus only to have it reactivated a short period later, whereas many children, when they have convulsions for a number of reasons, fever being one of them, these convulsions are usually of short duration. They have one, and that's it. It is really a different bag of worms when you are talking about the population you are dealing with, so maybe this is one reason why the short-acting are popular in one element, but I think most neurologists prefer to use phenobarbital.

DR. AZAR: We also have to keep in mind--I tried to make this point this morning--that the UDMH and hydrazine both are respiratory depressants and we do not want to further depress respiration with the treatment and this was one of the thoughts in making the decision as to what barbiturate to try. It is generally felt that shorter acting barbiturates cause more respiratory depression than the sodium phenobarbital.

MAJOR THEODORE: Let me just add one more thing. Anyone that has convulsions is always prone to respiratory arrest. I am sure that if any of you have noted anyone with a convulsion you will certainly want to watch him under the most optimal conditions and anyone that has convulsions is watched in this manner and he just isn't given a drug and everyone sort of saunters away and isn't concerned any more. So, these people are going to be under very intensive care and observation and whether you want to use a short acting or a long acting barbiturate or whatever you want to use, these people are going to be watched closely, usually in a setting where respiratory support is going to be available. So maybe we are really quibbling about semantics.

DR. AZAR: I agree.

MR. BATTISTA: This is sort of academic, but if you were to go through your data and examine in terms of how many lethal doses, how many LD-50's could you protect against in an animal by giving the B-6 for the hydrazine or for the UDMH, what kind of numbers are we looking at?

DR. AZAR: I believe Dr. Back in 1963 reported the ED-50 of pyridoxine against two times the LD-50 of UDMH was I believe around 50 milligrams. He can correct me if I'm wrong on that.

DR. BACK: Thirty, I think.

DR. AZAR: He thinks also. This was published in 1963. The hydrazine I do not know, and I would like to see the work that I did duplicated, only using higher doses of these mixtures. This therapy may not work against higher doses. At this time I can't say that it will work or won't.

DR. DOST: In a strictly non-clinical situation I think that we see, and it has been reported elsewhere also, that the time at which the B-6 is administered is critically important. In other words, you have much better protection capability if the drug is administered at the time the convulsion begins as compared with the prophylactic use. Primarily, we find in strictly experimental conditions with rats, that we can protect against otherwise lethal situations if the compound is given right when the animal begins to convulse.

DR. THOMAS: We are not thinking of prophylactic use at all in this instance. Both Ken and I feel, in working with this compound for quite some time, that the likelihood of voluntary lethal exposure is "zilch". Nobody will stick around that long, if he can get out, to absorb a convulsive dose which is not necessarily lethal. You get nausea first, and I would say that you have got plenty of time to keep the patient under observation. The first time he vomits, grab the pyridoxine and inject it. But I would not get upset until I see some clinical signs coming up.

DR. AZAR: Priorson has reported six cases of human exposure to the inhalation of Aerozine -50 and these individuals did suffer from nausea, vomiting, weakness, and tremors and they responded within twenty minutes to pyridoxine hydrochloride. I'm not certain of the dose. I believe it was approximately 400 milligrams total dose. Two of the individuals required steroid therapy to treat evidences of pulmonary edema that developed. All six individuals survived. However, remember that the analog of pyridoxine, pyridoxal, has been shown to accentuate the toxicity of certain hydrazine analogs.

DR. BACK: The point here is that the rat is not the animal to look at as far as the choice of antidote goes because the rat is most susceptible to pyridoxamine and much less susceptible to pyridoxine as an antagonist, whereas the mouse is ten times more sensitive to pyridoxine than he is to pyridoxamine, and so is the monkey. You

can't abort convulsions in the monkey with pyridoxamine--only with pyridoxine. You can abort it, but you have to go to tremendous doses like 250 milligrams per kilo, which is way out of proportion to the amount that it takes. It only takes 25 mg/kg of pyridoxine where it takes 250 mg/kg of pyridoxamine. So, I think humanwise we have to guess that pyridoxine is the drug of choice; besides that, it is the one on the market. Pyridoxamine is not available. The other thing that I think is important is that pyridoxine will abort the convulsion after it has started. If you have the first convulsion and you are likely to have another one and you give pyridoxine, you can abort the second one. You will just not have it. So it is good at any time during the exposure.

LT. COLONEL KAPLAN: I believe you said that the pyridoxine-treated surviving mice did not show fatty livers. Since the maximum deposition of triglyceride in the liver after a single dose of hydrazine occurs during approximately the first 16 hours and then returns to normal at approximately 24 hours, did you check any of the earlier ones, or were these animals that had survived for several days?

DR. AZAR: These were animals that had survived a week.

LT. COLONEL KAPLAN: Oh, I see.

DR. JACOBSON: I wonder if I could get you to speculate as to this apparent antagonism of dimethylhydrazine toxicity by hydrazine. I wonder if you think it has any practical significance, and if you have any evidence or ideas on the mechanism?

DR. AZAR: Is Major Carter here? I would like to point out this is strictly conjecture, but one of the possibilities is that there might be a simple chemical interaction of the UDMH and Hydrazine in solution, and Major Carter (this is strictly preliminary data) has tried injecting UDMH/hydrazine in separate areas of the peritoneum, and he continues to see the apparent antagonism. I could also speculate that this may indicate a different mechanism of action of the UDMH and hydrazine than that which has been proposed by other investigators such as Weir and O'Brien. As you know, these compounds have different effects on the animals. The UDMH convulsion is more a popcorn-type convulsion, with the animals bouncing violently against the cage and the hydrazine poisoned animal is more depressed and has a rolling type convulsion. It may be that the hydrazine in low doses is a depressant and that this is counteracting or antagonizing the effect from the UDMH. This is strictly conjecture though. The practical significance of this I have no comment on.

MR. WANDS: I wonder if I might throw a question at Ed Vernot at this moment about  $NF_3$ . You had two species whose intercept on your log-log plot came out at the origin, although it wasn't really an origin on the log-log plot, but you also had two species with a positive intercept. Does this indicate a difference in the mechanism of the action in those two species?



MR. VEKNOT: Well that wasn't a log-log plot. It was a linear plot of the concentration versus the reciprocal of the time, and you get linearity out of that kind of a plot if you have your LC-50 times time equal a constant. If you have LC50 times time equal a constant you also go through the intercept. If you have that classical CT relationship, you also get a linear log-log plot because the log of the reciprocal of the time and log of the concentration will turn out to be proportional. However, if you get what we got in the case of the mice and in the case of the monkeys, an intercept, you won't get a linear log-log plot because there is no way you can take a log of a sum, one of which is variable, and get linearity. If I had the second two on log-log plots, it would not have turned out to be linear.

I'm kind of a kindergarten pharmacokineticist in that I'm interested in this thing, and there is a way to rationalize that intercept. You have to postulate that the rate of distribution of the methemoglobin, or whatever the toxic material is, is constant, that is the rate is a constant, is not dependent on the concentrations of the material, and this sounds great except that it also appears that this is true for the dogs and the rats as well, and yet it goes through the origin. So I really don't know. I'm quite interested in what the real reasons for this might be. I don't know what they are.

MR. MOBERG (Aerojet-General Corporation): I would like to ask a question on the hydrazine/UDMH. In any of the body tissue or functional analyses, did anyone look for dimethylnitrosoamine as one of the oxidation-reduction products of UDMH, as one of the secondary reactions? Of course we know a little of the toxicity of that material. Has there been any examination along that line?

DR. BACK: Well, I don't really want to field it, but no one has ever really looked at nitrosoamine. However, the toxicology of the compound is one of the strongest hepatotoxins that we know of and if UDMH went to a nitrosoamine you would expect to have livers that were no longer functional and you don't see this with the compound, so I have to conclude that there was none.

MR. MOBERG: I don't know of any attempt to look for dimethylnitrosoamine in any biological system to which dimethylhydrazine had been administered but I know that this search for the back reaction has been carried out in non-biological systems. I'm not completely familiar with all the data or all the methods. My impression is that no evidence of the back reaction has been found, by back reaction I mean the oxidation of dimethylhydrazine to dimethylnitrosoamine, but I believe that the analytical chemistry on the dimethylnitrosoamine is not sufficiently sensitive to permit the conclusion I just suggested to be stated with confidence. Can anyone support me or refute me on that? It is a matter of considerable interest in many fields, in the pesticide field, in the preservation of fish where amines are treated with nitrate, as well as in the propellant field.

DR. LAWTON: Can we go back to the carbon monoxide paper? In the people working with low levels of carbon monoxide, do you find a change with activity, that is, when a person is working hard under an emergency situation or under a physical exertion, do they pick up a higher level of carboxyhemoglobin at a greater percentage than they would in resting conditions?

MAJOR THEODORE: I can't answer your question as far as exercise is concerned, but I do know by increasing the rate of inhalation you will increase your rate of carbon monoxide diffusion across the lung blood barrier, if you will--I'm using this term very loosely now. So it is dependent upon alveolar ventilation, and the rate of carboxyhemoglobin formation is a function of concentration and a rate of uptake.

DR. BACK: If you are not at equilibrium.

MAJOR THEODORE: Yes, if you are not at equilibrium. Once you've reached equilibrium regardless of what you do to ventilation you just can't surpass that limit. But I'm talking in terms now of initial exposure and maybe I'm giving myself a lot of room here, up within 24 hours, and this will be dependent upon not only minute ventilation but also on cardiac output, or better, pulmonary blood flow. But once you've reached equilibrium regardless of what you do to your ventilation you are not really going to change the carboxyhemoglobin level. It is in the initial phase that I am referring to. Is this what you are talking about?

DR. LAWTON: Yes.

MAJOR THEODORE: With acute exposure, yes it will be dependent upon minute ventilation and cardiac output, both of which will be increased with exertion.

DR. BENJAMIN: I would like to raise another problem that bothered me for quite some time, and which I brought up repeatedly here before. That is the problem of standardization of units. If you talk about parts per million we have difficulties at altitude. Parts per million is related to total number of molecules present in the atmosphere. Really it doesn't make much sense because most toxic factors are independent of the total number of atmospheres present. They are related to the total number of molecules present per given volume. If you express it, on the other hand, as it was expressed several times this morning, in milligrams per given volume, then we do not deal with the toxic factor involved. The actual factor as it affects the living system is a molecule and not a milligram. We have to convert it in order to get meaningful comparative data. Therefore the National Academy recommended that we express it in terms of a unit that corresponds to parts per million but is independent of the atmosphere and that is micromoles per 25 cubic meter which gives the same number as parts per million but it makes it independent of any other atmospheric component present.

QUESTION: Did you say per 25 cubic meters?

ANSWER: Yes.

DR. JACOBSON: I'm astonished why it would be 25 rather than 24.4. I think the figure that they are striving for there is the volume of a "Gram-Mole of air at 25 degrees Centigrade and 760 millimeters of mercury". Am I correct?

DR. BENJAMIN: Yes.

DR. JACOBSON: If my memory is correct that figure is 24.4.

DR. BENJAMIN: I think you are correct but the recommendation was 25.

FROM THE FLOOR: Twenty-five is easier to say.

DR. BENJAMIN: You are right. It should be 24.4.

QUESTION: Is that right, Mr. Wands?

MR. WANDS: Yes. That was purely a convenient rounding off that one of the committee on the Space Science Board utilized for their recommendations for long term space flights. We talked of this extensively last year.

FROM THE FLOOR: I'm curious if we can round off  $\pi$  to three and one-half?  
(Laughter)

MR. WANDS: I think it might be of interest to this group to give you a very preliminary report on some work which some of the Navy people have been doing. They are not here to defend themselves or to speak for themselves. I don't even have authorization to speak for them, so this is purely rumor that I am spreading right now. Treat it as such. They have been doing some hyperbaric studies with carbon monoxide and they are finding that the toxicology of carbon monoxide is strictly proportional to the pCO, the partial pressure of carbon monoxide, regardless of the total ambient pressure.

DR. CULVER (SysteMed Corporation): Looking at carbon monoxide, as you go to altitude, you have to take into account the shift in the dissociation curve. I find Ralph Wand's rumor most interesting, therefore. How do you explain that, Ralph?

MR. WANDS: I'm sorry, I didn't get the total question, but I should probably qualify it that this rumor that the toxicity of carbon monoxide is strictly proportional to the partial pressure is true so far only in the presence of a constant partial pressure of oxygen.

DR. THOMAS: One more last thought about carbon monoxide. We are not only going to ask the committee to review the NF<sub>3</sub> EEL's but also ask the committee to take a look at our data on the human performance studies during three hour single exposures all the way up to 250 parts per million because we think there is a serious question about the recommendation to set a CO limit around 15 PPM and even probably lower. This is especially important because, correct me if I'm wrong Ralph, 15 PPM wound up also as a recommended space cabin limit for exposure in the Space Science Board document.

MR. WANDS: I can't remember the number off-hand. We will certainly be anxious to see the data when it is available for us.

AMRL-TR-69-130

SESSION III

### HISTOPATHOLOGICAL EVIDENCES OF TOXICITY

Chairman

Dr. Frank M. Townsend  
Clinical Professor of  
Pathology  
University of Texas Medical  
School  
San Antonio, Texas

## HISTOPATHOLOGY AFTER EXPOSURE TO MIXED GAS AT 5 PSIA FOR EIGHT MONTHS

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### INTRODUCTION

This experiment is a continuation of studies in the evaluation of a mixed gas environment. It deals with the histopathology of dogs, monkeys, rats, and mice exposed to 68% oxygen and 32% nitrogen at 260 mm Hg (5 psia) up to periods of 320 days. A similar experiment has been reported previously (Patrick, 1967).

### METHOD

General experimental procedures and chamber operation for this experiment are presented elsewhere in the Conference Proceedings. Some of the animals that were exposed were submitted for histopathologic evaluation. Tissue samples were taken at Wright-Patterson Air Force Base and submitted for routine staining. Pathologic alterations were graded and the distribution and severity of lesions were noted for each animal. In addition, control and experimental animals were compared as groups for a subjective evaluation of any change.

Most of the animals were kept in the exposure dome for 8 months and sacrificed. Seven animals (4 dogs and 3 monkeys) were kept in the exposure dome for 8 months, returned to ambient conditions for 3 months, and sacrificed at 320 days.

In certain dogs and monkeys, the left kidney was exteriorized so that sequential biopsies could be done. These results are reported elsewhere.

### RESULTS

Table I depicts the number of each species examined histologically.

#### Dogs

Table II lists histopathologic alterations noted in control dogs and those exposed to a mixed gas environment.

TABLE I  
ANIMALS EXPOSED TO MIXED GAS

	<u>CONTROL</u>	<u>MIXED GAS</u>
Monkeys	4	6
Dogs	6	8
Rats	10	10
Mice	10	10

TABLE II  
PATHOLOGIC ALTERATIONS IN DOGS

<u>NUMBER</u>	<u>DIAGNOSIS</u>
	<u>CONTROL</u>
1124	Hepatic Congestion Chronic Pyelonephritis, Focal
1128	Hepatic Congestion Tubular Dilatation
	<u>EXPOSED</u>
1209	Casts, Renal Tubules
1213	Bronchiolitis, Focal Chronic
1214	Bronchiolitis, Focal Chronic

Monkeys

Table III lists histopathologic alterations noted in monkeys.

### Rats

Ten control rats and 10 exposed to a mixed gas environment were examined. All showed moderate to severe chronic murine pneumonia with an interstitial as well as a peribronchial component. This change was qualitatively and quantitatively similar in both groups. Focal chronic inflammation was noted in the kidney in 3 control rats and 1 exposed rat. Similar changes in the liver were noted in 2 of the control group and 1 in the experimental group.

### Mice

Ten control mice and 10 mice exposed to the mixed gas environment were studied. Table IV depicts the incidence and severity of changes attributed to chronic murine pneumonia.

TABLE III  
PATHOLOGIC ALTERATIONS IN MONKEYS

NUMBER	DIAGNOSIS
	<u>CONTROL</u>
1126	Lung Mite Cyst, Focal
1127	Lung Mite Cyst, Focal Pyelonephritis & Fibrosis (Exteriorized Kidney)
	<u>EXPOSED</u>
1211	Lung Mite Cyst, Focal
1212	Chronic Bronchiolitis, Focal
1220	Lung Mite Disease with Focal Chronic Pneumonia

TABLE IV  
MURINE PNEUMONIA IN MICE

<u>DEGREE</u>	<u>NUMBER OF ANIMALS</u>
	<u>CONTROL</u>
Severe	3
Mild	5
Slight	2
	<u>EXPOSED</u>
Severe	3
Mild	3
Slight	4

### DISCUSSION

This study is similar to one which was reported in The 3rd Annual Conference Proceedings of 1967. Endemic disease was similar in that experiment in control and exposed animals and no lesion was noted which could be attributed to the mixed gas environment. The findings in the present study also indicate that there is no lesion using light microscopy which can be attributed to the mixed gas environment.

It is well known that monkeys exposed to 100% oxygen at 750 mm Hg develop a characteristic and sometimes fatal exudative and proliferative pulmonary lesion (Kaplan, 1969). Clinical and pathologic alterations develop during the first week of exposure. Marked clinical improvement is sometimes noted in survivors after exposure day 9. Pulmonary fibrosis was described in surviving monkeys.

In another study animals (rats, mice, dogs, monkeys) were exposed to 100% oxygen at 5 psia for periods up to 235 days (Hagebusch, 1966). Subtle pulmonary pathologic alterations in dogs were apparently related to this environment.

In the present study pulmonary pathologic alterations which occur with oxygen toxicity were notably absent. It is pertinent to add that endemic pulmonary disease in dogs and monkeys was absent or minimal. In rats there was moderate to severe chronic murine pneumonia in all animals, and in this species pulmonary lesions from another agent might have been obscured. In mice, chronic murine pneumonia was similar in control and exposed groups.



In 8 dogs and monkeys one kidney was exteriorized for sequential biopsies. Scarring and chronic inflammation were noted in some of these, and this will be discussed in another paper.

In other tissues there were sporadic changes in both control and exposed animals. No lesions were noted which could be attributed to experimental conditions.

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2. Kaplan, H. P., et al; "Pathogenesis and Reversibility of the Pulmonary Lesions of Oxygen Toxicity in Monkeys"; Laboratory Invest., 20:94-100, January, 1969.
3. Patrick, R. L.; "Pathology of Animals Exposed for 240 Days to a 5 psia and 70% O<sub>2</sub> and 30% N<sub>2</sub> Atmosphere"; AMRL-TR-67-200, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, December, 1967.

## DISCUSSION

DR. COULSTON: I don't have any questions but I would like to make a few remarks. Let me ask first, in the early pictures of the lungs you showed where I asked how it was fixed--aren't you a little concerned with perfusion techniques of fixing where you make the alveoli balloon up, look bigger than they are? They looked so nice.

DR. PATRICK (Laboratory for Experimental Biology, St. Louis): You play both ends against the middle on this score. I prefer to see them both ways, perfused and non-perfused. If I must make a choice between one or the other, in large animals, dogs and monkeys, I would select the perfused lungs for this type of study, but it depends upon what you are looking for. If you are doing an acute experiment where you were looking for pulmonary edema and fibrin in the alveolar walls, early acute changes in the bronchi, my answer would be completely different. So what you do, again, as you well know, is dependent upon what you are looking for and under what circumstances.

DR. COULSTON: That is a good answer. I agree.

Now I would like to make a remark, particularly in reference to the mite in the lungs of the monkey. Some of you may remember that three or maybe four years ago at a meeting here where I was in the Chair, we had a very eminent veterinary pathologist who got up and said the monkey is absolutely worthless--the Rhesus monkey was so full of mites and tuberculosis that you could never possibly use a Rhesus monkey for these kinds of studies that you reported. This myth has been spread again, forgive me, amongst certain groups. It was reported the same way at a meeting last year of the Experimental Medicine and Surgery, about using primates, where I again took another man apart on this very same thing, and I'm delighted to see your remarks, that the mite is not a problem in interpreting lung pathology in the Rhesus monkey, provided you have a good Rhesus monkey colony, and I will reaffirm that.

DR. PATRICK: In amplifying what you say, I've only seen tuberculosis once in a monkey and that is when I was a resident and someone had a lung they wanted me to examine from a monkey, and in that first year of residency I thought I was looking at tuberculosis. I was looking at a parasitic disease. I've never seen a case of tuberculosis in a monkey, which means I guess that we humans don't carry them much any more.

DR. TOWNSEND: Dr. Patrick has had long experience in this field, as many of you know, and it is obvious that he knows of what he speaks. The lung in any animal, at least with the light microscope, is a difficult organ. In my work I see a lot of lungs and am aware of the multitude of difficulties that they present. I think a later paper this afternoon will give you some insight as to what other techniques may do, but in interpreting any of these lesions, it reminds me of the story about east Texas where the traditional eastern tenderfoot was being shown some land, and it was down in one of the East Texas river bottoms and it was in the dry season, and the real estate agent was showing him through this property, and one could plainly see on the trees where the water marks were from the previous flooding. And the Easterner finally commented on this, about what were those marks on the trees. The real estate agent said: Well, that's a lot of these razor-back hogs from Arkansas come down here and rub up against the trees. As they progressed on deeper into the swamp the marks got higher, and after it was all over, the real estate agent was anxious to close the sale and asked the Easterner if he would be interested in buying the land. He said: No, but he sure would be interested in some of those hogs. So some of this morphology that we see we have to interpret in the light of what we are looking at and what it may be.

## STANDARDIZATION OF NECROPSY AND HISTOPATHOLOGY TECHNIQUES

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I chose my subject for several reasons. Most of you are not pathologists and sitting through a long series of pictures of lesions might be of limited interest. Also, you have had a lot of "This is what we did and this is what we found". This will also give me an opportunity to give investigators a small insight into some of the problems that pathologists must deal with to provide you with the data upon which to evaluate the results of your experiments. I am going to cover some of the basic techniques as practiced at this Laboratory to solve such problems as quality control of laboratory rodents and tissue fixation techniques.

As was brought out so clearly in the preceding paper, chronic murine pneumonia and otitis media are big problems in a laboratory involved in inhalation exposure. They can completely negate the possibility of interpreting results. We are attempting to eliminate it from the animals with which we work by augmenting our quality control program. Otitis media is completely silent clinically. The old diagnostic method, holding a mouse by the tail and spinning him, will give you a dizzy mouse but no information as to how much middle ear infection is present in the colony (Olson, 1968). When rats or mice are received at the laboratory and before they go on test, we necropsy ten of them and collect tissue from five of them for histopathologic examination. We take five major organs and a slice of the head through the bulla tympanica to check on the presence of middle ear infections. The head is removed; as much as possible of the excess bone, muscle, and skin is cut away; and the head is fixed in formalin for three days. It is then decaified overnight. The technician cuts a thin slice, the plane of which must pass through both external ear canals. After being washed in running water for three hours, it is processed like any other tissue. Figure 1 is a head with one infected bulla tympanica that can be diagnosed only under the microscope. On the opposite side is a badly infected middle ear. We are using sections of tissues prepared this way more and more because many structures such as striated muscle, the middle and inner ear, pituitary gland, bone marrow, bone, brain, salivary gland, and nasal pharynx can be screened for disease. The brain in figure 1 also has lesions. In figure 2 the semicircular canals of the inner ear are filled with pus but the animal showed no evidence of vestibular malfunction. Figure 3 is the lesion from the brain of the same animal as in figure 1. The brain is not often screened for disease; however, it is as important as any other organ and one should know what diseases are being purchased along with the animals. Figure 3 shows one of the small lesions

in the Central Nervous System (CNS) and figure 4 shows the causative organism, Nosema cuniculi. This is also usually a clinically silent infection. It is very common in rabbits and is observed quite often in the mouse, but not quite so often in the rat (Garner, 1967). This infection came from the vendor and it might now be in our holding facilities; however, we know what we have to deal with.

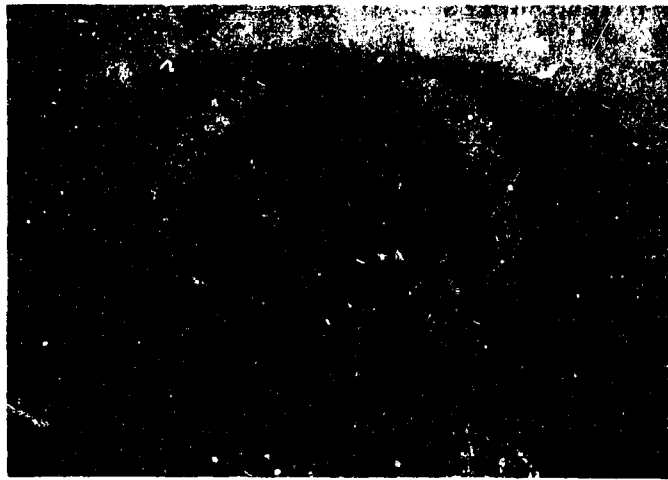


Figure 1. CORONAL SECTION OF THE HEAD OF A RAT THROUGH THE BULLAE TYMPANICA SHOWING A PURULENT EXUDATE IN ONE CAVITY AND THICKENED MEMBRANES IN THE OTHER. Dark spots in the cerebral hemispheres are granulomas caused by Nosema cuniculi. (H & E x 4)

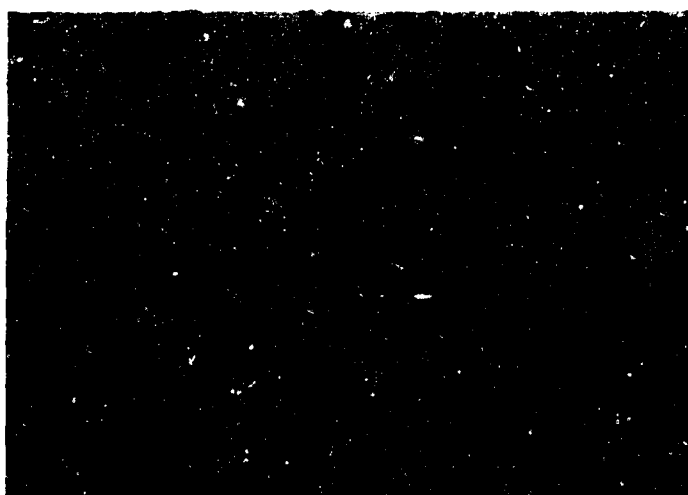


Figure 2. INNER EAR OF A RAT SHOWING PURULENT EXUDATE IN THE SEMI-CIRCULAR CANALS AND AN UNAFFECTED COCHLEA. (H & E x 100)

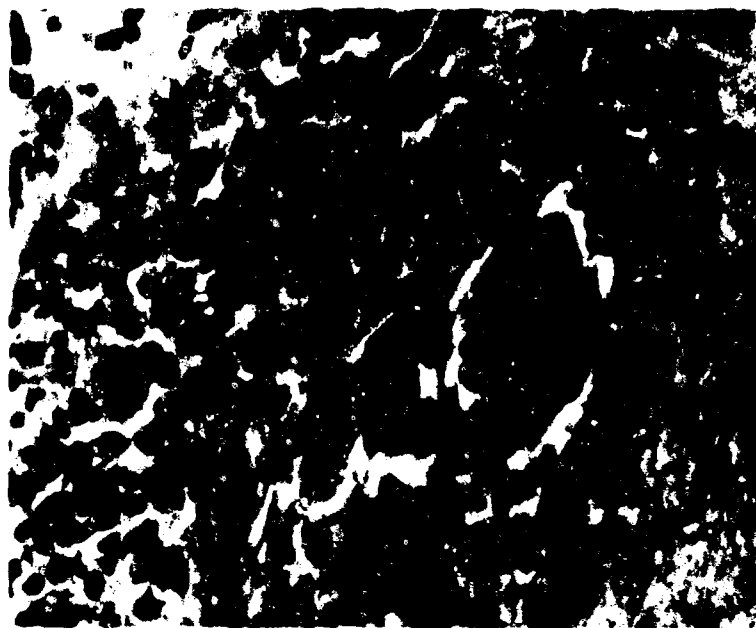


Figure 3. GRANULOMA IN THE CEREBRAL CORTEX OF THE ANIMAL SHOWN IN FIGURE 1, CAUSED BY NOSEMA CUNICULI. (H & E x 400)



Figure 4. PSEUDO CYST OF NOSEMA CUNICULI FROM THE BRAIN IN FIGURE 1.  
(McCallum-Goodpasture x 1000)

In going over these rats for quality control, we have to look at a lot of lungs. I do not like to screen any lung for disease that has not been infused, particularly in rodents. Figure 5 is an infused lung of a mouse. One can see how all of the tissue is standing out. The mucous membranes are very prominent and easily seen. Figure 6 is a mouse lung that has not been infused. It is as completely normal as the previous lung but it was allowed to collapse. We took particular care not to push this together. We handled it carefully and dropped it in formalin where it floated on the fluid and fixed. You can see lumps of tissue that become very similar to some of the lesions you saw in the previous presentation. Figure 7 is one of the collapsed areas on higher magnification. I have seen lesions like this called interstitial pneumonitis and atelectasis. This is a completely normal lung but I don't know how you could diagnose it as such with complete assurance. Figure 8 is an infused lung at increased magnification in which individual alveolar walls are apparent. You can easily make an estimate of any change that has occurred. This is what I like to look at, and I haven't noticed that we get much artefactual alveolar emphysema produced by the technique which we use.

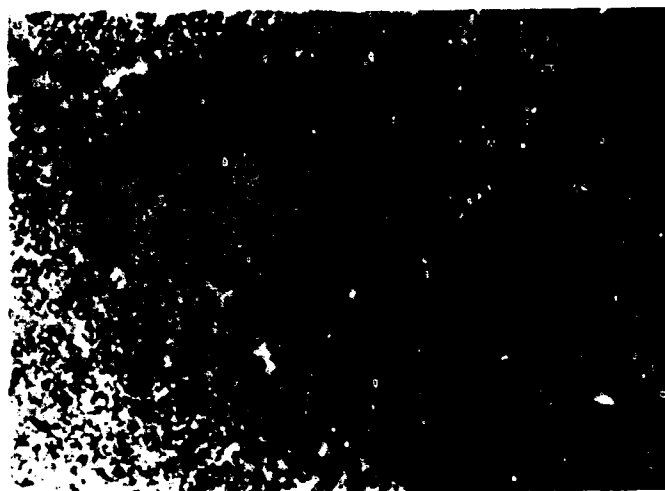


Figure 5. MOUSE LUNG DISTENDED WITH FORMALIN PRIOR TO FIXATION.  
(H & E x 40)

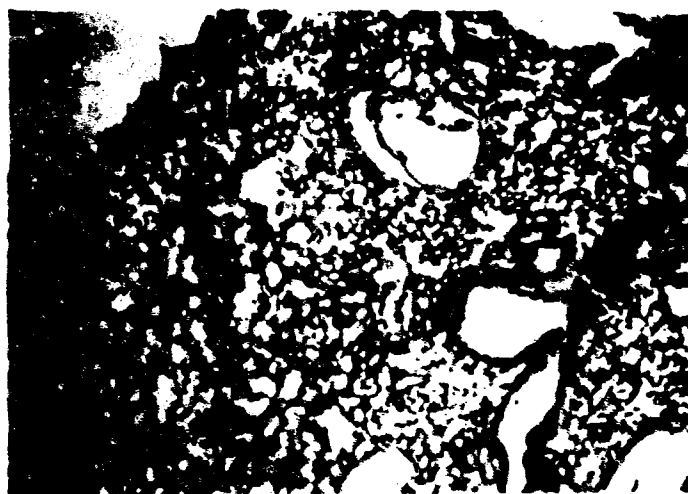


Figure 6. MOUSE LUNG ALLOWED TO COLLAPSE PRIOR TO FIXATION.  
(H & E x 40)



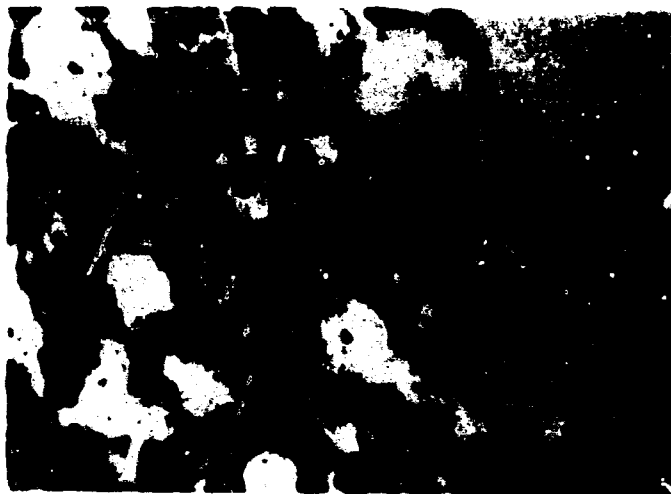


Figure 7. COLLAPSED ALVEOLI OF LUNG SHOWN IN FIGURE 6.  
(H & E x 100)



Figure 8. CLOSEUP OF ALVEOLI OF LUNG INSTILLED WITH FORMALIN PRIOR  
TO FIXATION. (H & E x 100)

Figure 9 is a thickened alveolar wall from a resolved pneumonia in an infused lung as seen under polarized light, demonstrating how easily the increase in size is seen. When killing many animals, instilling formalin into lungs is a time consuming process and it takes two additional men in the necropsy room but I think it is worth it. We open the animals and do a gross necropsy with the lung collapsed; if there is significant emphysema we see it, and we can also judge generalized vascular problems. We then cannulate the trachea using an 18 gage needle that has been dulled; put a string around it and, with a syringe, instill the lung until it stands where it normally would in the closed thoracic cage. We do it fast but it works very nicely for us. When the lung is expanded we tie it off, put the lung in a jar with the string over the edge, put the lid on the jar, and turn it upside down. Because of residual air in the lung it floats and fixes under the fluid. There is another advantage to inverting the bottle. We have found that tissue will sometimes stick together in the bottom and fix poorly. When the bottle is inverted, these masses of tissue are broken apart. We would like to expand all of the lungs with formalin but on the rat lungs we are taking organ weights. It is very difficult to expand the lung when you have to cut out the heart, thymus, esophagus, and a lot of the trachea to get an accurate weight. We worked on this a long time and, when the last group of rats was killed, 95% of the lungs were successfully expanded.



Figure 9. ALVEOLAR WALL THICKENED WITH COLLAGEN FIBERS SHOWN UNDER POLARIZED LIGHT. (H & E x 100)

In the necropsy room, one of the most difficult things to fight is postmortem degeneration. Good tissue fixation does not receive the attention it deserves, perhaps, because of the necessity to become more sophisticated in our experimental approaches. During my time at the Armed Forces Institute of Pathology, I saw some good experiments where there was so much postmortem degeneration that the fine changes in the tissue could not be interpreted. It is embarrassing to have to tell the investigator that he neglected such an important detail.

There are also other things that can affect tissue, keeping in mind that the pathologist is often months away from the necropsy and has to relate subtle changes to an experiment with which he has often had no part. Figures 10, 11, and 12 show examples of one of the many problems that are directly associated with fixation. I used the domestic pig because what I want to demonstrate is shown beautifully in this species. Figure 10 is liver from a pig that has not eaten for 8 to 12 hours. It is normal liver, well fixed. Compare figure 10 with figure 11. Notice how the hepatocytes are swollen, the cytoplasm is vesicular and filled with irregular, clear spaces, and the nuclei float in the middle of the clear space. This is also perfectly normal liver from a well-fed pig fixed immediately after death. Figure 12 is liver from a pig that had eaten but the fixation of tissue was delayed 30 minutes. Enzymes within the liver have digested the glycogen and it is gone. The cells are no longer swollen. These slides would be very difficult to interpret if the information as to eating and fixation were not available. This is an example of why necropsy techniques should be standardized. These are very simple things but they have to be done. A lot of time is involved in standardizing these procedures hoping to eliminate as many artifacts as possible. Animals are fasted, and are anesthetized as we necropsy them. Even though we have to take organ weights, the tissues are in the bottles within no more than 10-15 minutes.

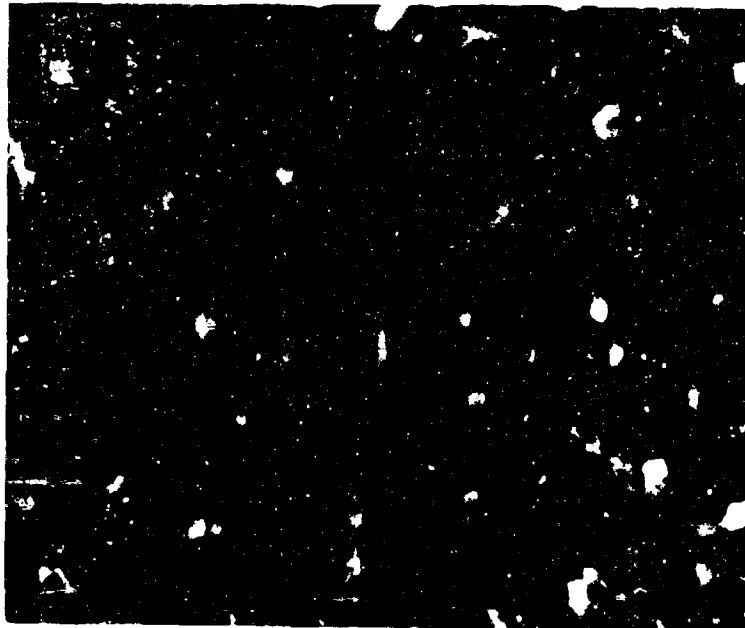


Figure 10. LIVER FROM A PIG THAT HAD BEEN FASTED 12 HOURS PRIOR TO DEATH. Tissue was fixed in formalin immediately. (H & E x 100)

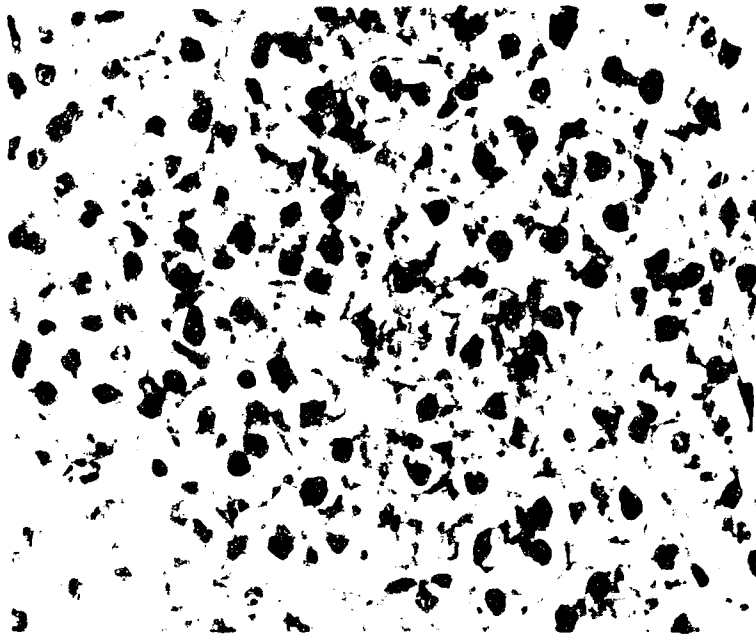


Figure 11. LIVER FROM A PIG THAT WAS FED FREE CHOICE UNTIL DEATH. Tissue was fixed immediately in formalin. (H & E x 100)

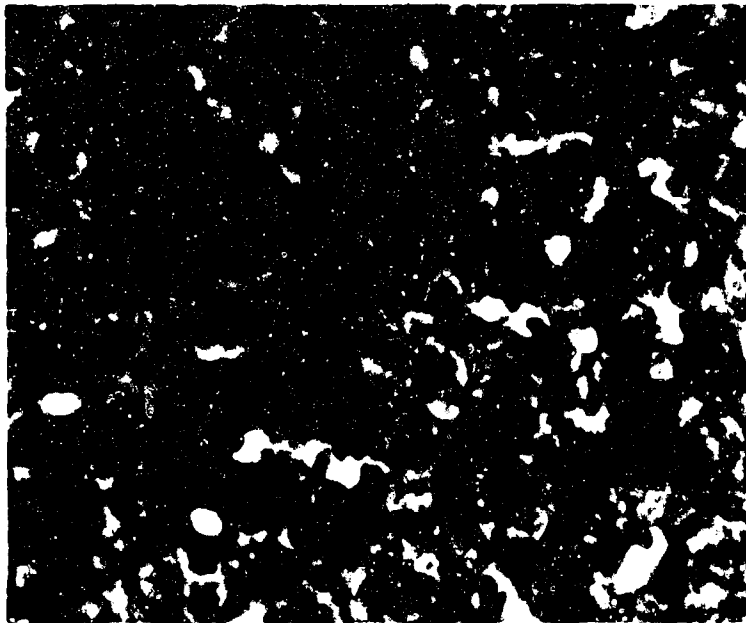


Figure 12. LIVER FROM A PIG THAT HAD BEEN FED FREE CHOICE UNTIL DEATH. Fixation of tissue was delayed 30 minutes. (H & E x 100)

There are also problems in clinical pathology. The means of the hematocrits of our dogs are 5% higher now than were reported in a paper presented by Robinson and Ziegler (1968) from this laboratory two years ago. The technicians often complained that the blood from some of the dogs was so thick that they had difficulty drawing it into the syringe. Some investigators had even cast aspersions upon the accuracy of our laboratory procedures. In searching for a reason for this increase in red blood cells, two factors became apparent. The source of dogs had been changed and many of the new dogs seemed excessively frightened and wildly fought restraint. The dogs from the new source were also young healthy beagles and the care and holding facilities were identical in every way to those used several years ago. We decided to test the possibility that panic and excitement were contributing to the surprisingly high hematocrits.

Four old dogs that were virtual pets were chosen. Blood samples were taken after gentle restraint, tranquilization with chlorpromazine, surgical anesthesia with pentobarbital, and fright caused by noise and rough handling. Unfortunately, the dogs were so domesticated that they did not respond to the attempts to frighten them; therefore, injections of metrazol, a strong central nervous system stimulant, were given to the point of convulsions. Significant increases in hematocrit and white blood cells were seen only after metrazol injections. The only other clinical test to change was total protein.

Armed with this preliminary information, we designed a more complete study using 10 randomly-selected, newly-acquired beagle dogs. Two cc blood samples were obtained after gentle restraint, chlorpromazine tranquilization (at 1 mg/lb) surgical plane anesthesia with pentobarbital, manually frightening the dogs, and after metrazol. A minimum of three days was allowed between blood drawings. The results are given in table I. The experiment was repeated in monkeys and the results were listed in table II.

TABLE I  
MEAN VALUES OF 10 NEW DOGS

	Quiet Restraint	Chlorpromazine	Pentobarbital	Manual Excitement	Metrazol
Hct (Vol %)	45	36	32	45	49
Total Protein (gm %)	6.0	5.3	5.4	6.4	7.0
WBC ( $\times 10^3$ )	15.2	13.5	9.5	18.7	17.9

TABLE II  
MEAN VALUES OF 10 NEW MONKEYS

	Quiet Restraint	Chlorpromazine	Pentobarbital	Manual Excitement	Metrazol
Hct (Vol %)	39	38	34	41	41
Total Protein (gm %)	7.8	6.9	6.6	7.9	8.1
WBC ( $\times 10^3$ )	10.1	8.4	7.7	12.2	11.3

Before coming to any conclusions, it must be clearly stated that this is preliminary data and certainly not adequate to support definitive statements. But it is entirely adequate to make a few generalizations and to point out some problems in interpreting clinical values in laboratory animals. In the old dogs, the hematology values for quiet restraint, tranquilization, anesthesia, and manual excitement were essentially the same. These old dogs were frightened but did not show the panic of the new never-before-handled dogs. This difference is shown in comparing the similarity of values between the quiet restraint and the chemical and physical excitement with the values obtained when the new dogs were chemically restrained. Indeed, there is really very little difference in the HCT, WBC, and total protein between just restraining the new dogs and causing them to convulse. The values obtained for the monkeys are similar but not as dramatic as those in dogs.

Solving this problem is far more difficult than defining it and I do not have an answer. We collect baseline clinical data from our dogs and monkeys every two weeks and the "normal" values for our animals are based on these values which reflect any "panic effect". This procedure also acquaints the animals with restraint and venipuncture. However, any new experimental procedure could cause a "panic reaction". As long as we are tied to traditional clinical tests such as the CBC we will have to contend with physiologic interference with our results. Perhaps it would be preferable to measure blood and plasma volume rather than venous hematocrit, since these are more stable and not affected by the mobilization of sequestered cells during transient physiologic states.

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## DISCUSSION

MAJOR MAC KENZIE (Aerospace Medical Research Laboratory): I might add, in perfusing these lungs we were not doing morphometric studies.

DR. CAMPBELL: I wonder if you would review the hematocrits again for the conditions that were involved. I had a little trouble with the chart.

MAJOR MAC KENZIE: The baseline value for dogs was 45 percent hematocrit. When tranquilized, --this is the average of ten dogs, 36 percent. Under the surgical anesthesia from pentobarbital, 32 percent. When purposely excited, 45 percent. Under the influence of metrazol, and this was taken within ten minutes of the reaction from metrazol, it was 49 percent.

DR. MAUTNER (Mount Sinai Hospital, New York): This is a question on the same slide which you just showed. All three parameters seemed to be moving in the same direction. The values are roughly comparable. As a guess, could it be the state of hydration or a question of the blood volume?

MAJOR MAC KENZIE: No, these were big robust beagles. Only 2 ccs were taken and there were several days in between this. This is not a very large blood loss for a beagle and there were other parameters that did not change.

DR. MAUTNER: Another point, while I am talking, in the human of course, we don't have the opportunity to standardize conditions quite as you do with the animals, and we have had to adjust ourselves to rather marked variations in appearance at autopsy which adds to your list perhaps, the disappearance of polymorphonucleocytes when you compare a patient who has had a liver-kidney biopsy shortly before death and then we have autopsy section and the polys have disappeared.

MAJOR MAC KENZIE: We've run into the same problem in diagnostic work in veterinarian medicine, only it is worse because a cow lies out in the pasture for four or five days before they find them.

DR. WEIBEL: I have a few comments, of course, which are related to the lung. First of all, I'm a little worried about this business of murine pneumonia because we don't have this problem in Europe. I just don't know why. We also have the Sprague Dawley rats, all sorts of rats, but we rarely have a case of murine pneumonia.



MAJOR MAC KENZIE: We have sources of rats that do not have this as a problem. I've seen something that might be murine pneumonia in breeding stock. We asked them to give us breeding stock so that we could see what they had in their colony and they did. They were very willing to. If they had refused I would have wondered why. There are clean colonies here. But, and I have to stress this, the words SPF mean nothing except that the animal costs more and does not eliminate the problem of middle ear infection or murine pneumonia.

DR. WEIBEL: Has this been systematically studied?

MAJOR MAC KENZIE: Yes and in Europe too. The latest papers are from Copenhagen. There is supposed to be a virus. I see very little doubt that there isn't a virus in the type of lesion that was shown here. There is also a PPLO that has been found numerous times that is involved with the interstitial pneumonitis and the middle ear infection, but these are not pure infections and they have never been studied in the gnotobiotic dog. So what we have seen is a secondary bacterial invader. We are also seeing the way the rat responds to a chronic irritation in the lung. I don't know if all rats with chronic murine pneumonia have the virus present. They might be suffering from some other type of irritant; I don't know. The amount of lymphoid tissue in the lung is open to question. I don't think that the small foci of lymphocytes at the junction of the major bronchi and trachea are important. I think they are just normally there, and I think with irritation these become larger and they become almost like the Peyer's patches in the intestines. Now what that irritation is; I don't think we should say this is a virus, yet.

DR. WEIBEL: Since your title has been Standardization of Techniques, and since we are expounding so much on the lung, I would like to expound on standardization of lung fixation techniques. Now first of all to get the term straight--If you say perfusion, you actually mean instillation or infusion of a fluid into a dead end system which does not go through, so it is not a perfusion. It is important to distinguish this term because we now have the means of really perfusing the lungs through the vascular system.

MAJOR MAC KENZIE: It's your fault. Aren't you the one that originated the "perfusion" of the lungs?

DR. WEIBEL: No, No, it has always been "instillation".

MAJOR MAC KENZIE: We got away with poor usage of words before.

DR. WEIBEL: Well, now I have noticed in your pictures that you used a syringe to inject this liquid into the lung. I think this is very bad. We have been doing quite a bit of work on ideal pressures to be used for instillation fixation of lungs, and it turns out that 20 centimeters of water at constant head pressure gives you a very uniform distension; with less you cannot do it. If you use 15 or 16 centimeters pressure you will get atelectasis areas and so on; and so you need to have that. Now this does not give you a collapse of the capillaries as you could see it in some of your

pictures there. The blood is not squeezed out of the capillary at 20 centimeters of water. I should perhaps mention that this appears to be somewhat species and size independent--we were quite surprised to find that for a dog we could also get away with 20 centimeters of water pressure. We usually fix in a closed chest and of course you have the high resistance of the chest wall and then we use a slightly higher pressure, 25 centimeters, maybe 30 centimeters. We try to keep it as low as possible. Now of course you get into a problem if you are looking at substances or changes that are in the alveoli, if you use instillation fixing. This is why we have now supplemented the technique by using vascular perfusion technique, and I am going to show in my paper now comparative electron micrographs of rat lungs exposed to pure oxygen fixed by instillation and by perfusion, classical perfusion techniques and you will see the difference. So I would strongly urge you to get away from the syringe because with your syringe you can exert a tremendous pressure and you can really rupture the lung. If you have a little resistance, like when the cylinder sticks, then you are just pushing it hard with your thumb and zip, off goes your lung and if you have a 10 cc syringe in a mouse lung this is about five times the volume of the lung.

Well, then, I would like to add another point which has to be standardized for experimental work at least, and this is the duration of anesthesia up to the point of necropsy. This is very, very important. We have run into this problem quite a number of times.

MAJOR MAC KENZIE: Thank you, Dr. Weibel. We are not unaware that the syringe is not the best system, but we are killing large numbers of animals, and so far this has been good. This is one of the things that I wanted to talk to you about.

DR. WEIBEL: You take a large jar. This is much faster, and it is easier than filling the syringe.

MAJOR MACKENZIE: Fine, good. Now as far as what we are using these lungs for--light microscopy and routine stuff--when they have murine pneumonia it doesn't make a lot of difference what you do.

DR. PATRICK: I have just one comment or two. I agree with you. I think that hydrostatic pressure should be used rather than a syringe. I would like to comment on your paper in general. I am pleased that you emphasized the point that we ought to know something about nature before we dicker with it, and this is really what you are saying, and I think too often we overlook this so I am exceedingly glad to see someone present a paper of this type, not only here, but in the country at large, in experimental circles.

DR. WEIBEL: Would you tie up that statement just a little bit more? It is too general for me to understand.

DR. PATRICK: No. (Laughter)

DR. COULSTON: I'm enjoying this very much. First of all, let me say, whether you call it perfusion or infusion, I think you can use a syringe. I have been using a syringe since 1934. Of course if you want to pick your pet species to work with, you work with a bird because you can never overpressurize the lung because you have air sacs which are a nice reserve for any excess pressure or fluid if you want to see lungs.

FROM THE FLOOR: You don't have chronic murine pneumonia either.

DR. COULSTON: But you've got other problems. But seriously, you are quite right to call it infusion of the lung and you are quite right to call it perfusion if you go through some of the vascular systems, or even through the cerebral vessels, if you wish. I think the distinction is clear, there is nothing new about this technique. I want to make it clear this was started by Maximal in about 1911 and has been used successfully for years by Bloom and his followers. But we did find in the early days in Chicago when I worked there, that it was wrong to perfuse the animal by an intravenous route for example--if for example you were going to study the red blood cells. Suppose you are studying malarial infection, you certainly don't want to infuse the animal. I think it is essential that the sinuses and sinusoids and capillaries of the animal show their true structure with the true cells, and particularly the spleen, is very important. Now this is not a criticism, this is just a comment. Now when you come to things like thorazine or pentobarbital causing problems, I would remind you that these compounds cause a very great cooling of the blood, particularly in an animal, they lower the blood temperature considerably--four degrees Fahrenheit sometimes, and this may be of importance here. I'm just throwing this out for what it is worth, but cooling is a possibility to be considered particularly in cellular elements. I want to compliment you, both of these papers are very pertinent for the everyday operation of the laboratory. Thank you.

MAJOR MAC KENZIE: Let's see if I can remember what you said. On the use of thorazine and pentobarbitals, all the animals had been around a long time and were used to being handled so this did not make a significant effect. It did when we gave them pentobarbital. There was naturally a blood temperature drop because it does cool. The thing that I found most interesting is the fact that even when we tried to excite them they didn't change, they were already as excited as they could get and they really didn't change that much when we gave them metrazol to the point of convulsion. This shows what psychology does in animals, it is simple but it really does affect the parameters that we measure. I won't go into that.

COLONEL STEINBERG: I'm glad to see somebody come up with this in dogs. We found the same handling phenomenon in rabbits and dogs, beagles in particular, but we found in handling rabbits, the first few times we bled them, the best thing to do was to throw out the data. The surprising thing that goes along with your metrazol work is of course that the rabbits still continued to get excited as you handled them but the blood chemistry and the hematology results level out. As a matter of fact, the biochemist that does these tests, we were threatened with all kinds of dire results because we found things that shouldn't have been knocked out: cholinesterase, BUN, and other liver function studies just go haywire.

FROM THE FLOOR: Enzymes?

COLONEL STEINBERG: Right, and yet your standards and controls are still working. We also found this true, as I say, in dogs. It happens in monkeys too.

MAJOR MAC KENZIE: Have you found lipemia?

COLONEL STEINBERG: Yes, but we found this was a good deal dependent on-- we don't raise our own rabbits, and it was how long we held the rabbits. If they come from the outside we tended to get more than if the rabbit was in our own lab after its conditioning period. We find lipemia now and then. We used to think that somebody slipped up and fed the animals. It has been shown, at least in the dogs and cats, that stimulation to the limbic system, particularly around the basal ganglion will produce lipemia in certain areas, and through a mistake that the investigator had made, they grabbed the wrong dogs, dogs that hadn't been stimulated, and they found lipemia in some of these, and they have been able to reproduce this as a fright phenomenon. Now this is fairly well known in many domestic animals but not to the point of visible chylomicrons circulating, and we have to apologize to some handlers now because I guess they didn't feed them.

There is something else that I would like to make a remark on: These things are all fine, but they are not worth anything unless you write them down on the clinical history in the necropsy protocol so that the man at the microscope has something to go back to.

DR. TOWNSEND: This whole area of standardization is one which could be pursued for hours, not only in experimental work, but in clinical chemistry, other areas where we see variations, particularly in specimens being shipped, the way they are taken, the type of anticoagulant, how soon the serum was removed from the clot, many other factors that really need a lot more study than they have had. One of the better studies came right from Dr. Patrick's Lab, as reported by Dr. Hagebush a few years ago, but this is still another story. For those of you who are not anatomists or pathologists or have not followed the story of the histology of the lung, years ago one of the many arguments that pathologists engaged in amongst themselves was the structure of the alveolus, the air sac of the lung, as to what type of lining cell it had, what was its anatomy, and with the advent of the electron microscope as a practical tool for laboratory study, a lot of this confusion has been cleared up.

## PULMONARY PATHOLOGY OF OXYGEN TOXICITY

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### INTRODUCTION

In our previous studies on the pulmonary effects of pure oxygen breathing at atmospheric pressure we have observed that endothelial cells of alveolar capillaries are the primary target of cytotoxic oxygen effects on the lung (Kistler, Caldwell, and Weibel, 1967). This observation, which has been confirmed since (Bowden et al, 1968; Kapanci et al, 1969), was puzzling because the endothelial cells actually compose the second cell layer met by the oxygen molecules diffusing from the air spaces; and the squamous epithelial cells are structurally very similar to endothelial cells. The question why these epithelial cells were not damaged in spite of direct exposure to oxygen, whereas the endothelial cells were largely destroyed remained unanswered. On the basis of observations of other authors (cf. Davies and Davies, 1965), it could be proposed that oxygen can exert its cytotoxicity only in presence of additional factors. If such factors are endogenous and can be supplied by the blood endothelial cells of alveolar capillaries could be regarded as the site of most intense interaction between oxygen coming from alveolar air and "additional factors" supplied by the blood.

Two sets of observations were of importance in elaborating the hypothesis at the basis of the present experiments.

- a) It has been observed that enzyme inhibition due to high oxygen partial pressures depends on contamination of the preparations by heavy metal compounds (cf. Davies and Davies, 1965). Furthermore, microsomal lipids are peroxidized under high  $O_2$  tensions only in the presence of additional factors, in particular iron compounds (L. Ernster, 1968, personal communication).
- b) Several workers have observed that high  $O_2$  tensions lead to a rapid damage of red cells, detectable, in part, by an increased osmotic fragility and by consequent hemolysis (Mengel et al, 1964; Danon, 1966; Bernardini, 1969). The mechanism at the basis of this erythrocyte damage is unclear, but some authors have speculated that it occurs by peroxidation of mem-

brane lipids resulting in alterations of membrane structure (Kann et al, 1967). This hypothesis is supported by the observation that deficiency in  $\alpha$ -tocopherol (vitamin E), a potent antioxidant, aggravates this effect (Kann et al, 1967; Carolla et al, 1968).

These findings led to the following hypothesis for the time-sequence of oxygen-induced pulmonary damage:

A primary damage of erythrocyte membranes in presence of iron compounds within the cell causes loss of these compounds into the plasma. If they are picked up by endothelial cells of the alveolar capillaries, their cell membranes will undergo damage. The resulting leakage of fluid into the interstitium (interstitial edema formation) may bring iron compounds into the neighborhood of epithelial cells. A subsequent damage of these cells on the basis of the same mechanism should then lead to alveolar edema.

The time sequence of the second part of this hypothesis has been demonstrated (Kistler et al, 1967; Kapanci et al, 1969). If the first part holds true there should also be a correlation between erythrocytic and pulmonary damage due to oxygen, as well in terms of quantity as of time sequence.

To test this part of the hypothesis two experiments were presently carried out:

- A. Normal rats were exposed to 98% O<sub>2</sub> at 760 mm Hg for up to 3 days; the development of hematological and pulmonary changes was studied.
- B. The same was repeated with rats fed  $\alpha$ -tocopherol deficient diet in which red cell damage should be exaggerated.

## MATERIAL AND METHODS

The experiments were carried out with albino rats from our local breed; they belong to the SPRAGUE-DAWLEY strain. For each experiment, controls and test animals were derived from closely related litters and carefully matched, at the outset, with respect to age, sex and body weight.

Before sacrifice the animals were deeply anesthetized with Hypnorm<sup>®</sup> (Duphar-Philips); one of the femoral veins was exposed and a blood sample of 1.5 ml was withdrawn always exactly 12 minutes after injection of the anesthetic. The lungs were then fixed by intratracheal instillation of 1.5% glutaraldehyde buffered with s-collidin to pH 7.4; the solution was adjusted to an osmotic pressure of 336 mOsm. After volumetry of the excised lung, tissue blocks were post-fixed with OsO<sub>4</sub> and block-stained with uranylacetate; dehydration in ethanol and embedding in Epon followed. Sections cut with diamond knives were stained with lead citrate and observed in a Philips EM 200 electron microscope.

Morphometry followed the preestablished scheme using a semi-automatic equipment and a computer for data analysis (Weibel et al, 1966).

The blood samples were immediately analyzed for hematocrit, total hemoglobin, plasma hemoglobin, and osmotic fragility; the latter was estimated by spectrophotometrically determining the increase in hemoglobin released into a graded series of NaCl solutions of 0.6 to 0.2%.

Exposures to 98% oxygen were done at 760 mm Hg in a closed environmental chamber (figure 1) which had the following characteristics:

- a) The chamber was made of a PVC-coated oil drum fitted at the flat faces with two removable Plexiglass windows by means of rubber gaskets. To permit removal of cages without decreasing the  $O_2$  concentration one end of the drum could be fitted with a plastic tent which was continuously flushed with  $O_2$ . The partial pressure of  $O_2$  did not fall below 710 mm Hg when the chamber was opened (ambient pressure is 710 mm Hg), and this lasted no more than one minute. This tent was fitted with arm inlets so that the animals could be anesthetized in the tent and kept at about 715 mm Hg of pure  $O_2$  until preparation 15 to 60 minutes later.
- b) The pressure was maintained at 760 mm Hg  $\pm$  2 mm Hg by a constant inflow of  $O_2$  from a tank at 2 l/min. supplemented by intermittent flow 10 l/min. for short periods; this was governed by a magnetic valve which was automatically controlled by a pressure sensor. A controlled leak was so calculated that the supply of  $O_2$  was adequate to meet the  $O_2$  consumption by the animals.
- c) Part of the chamber air was cycled through a bypass; a pump drove it through flasks containing silica gel to remove water vapor, LiOH to absorb  $CO_2$ , charcoal to eliminate odors, and two mechanical filters before flowing back into the chamber. Temperature was maintained at 20 to 25 C by first cooling the cycled air and then rewarming it by a heating coil governed by a temperature sensor in the chamber. Since both fresh oxygen supply and recycled air were essentially dry, humidity was controlled by a De Vilbiss humidifier regulated by an hygrometer.
- d)  $O_2$  partial pressure was continuously determined polarographically (Beckman Oxygen Analyzer),  $CO_2$  tensions by an infrared spectrometer (MSA-LIRA model 300) sensing the outflow leak; these parameters, as well as total pressure, temperature, and humidity were continuously recorded on a Philips Comprecorder 8400.

All control animals were kept in room air in the animal house at ambient pressure (about 710 mm Hg).

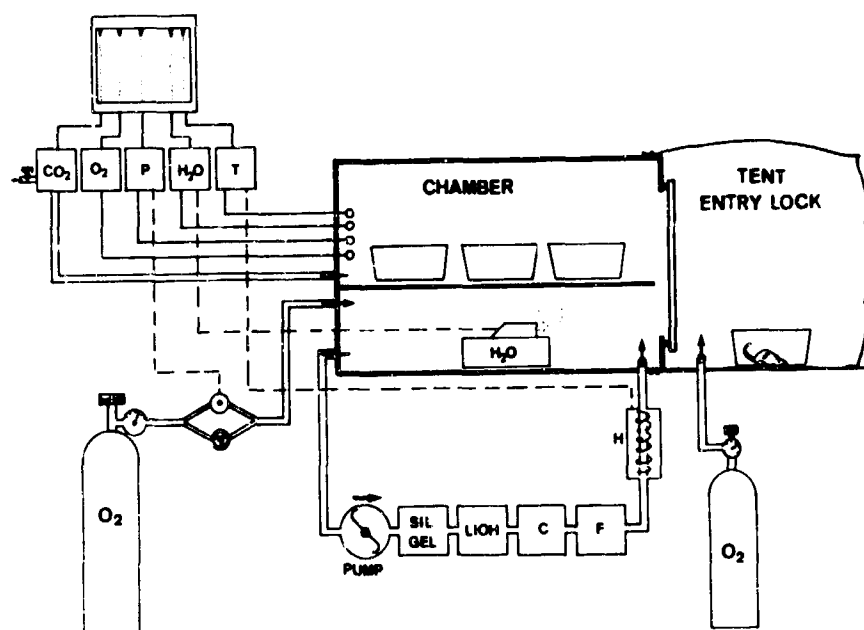


Figure 1. DIAGRAMMATIC REPRESENTATION OF EXPOSURE CHAMBER WITH TENT AS ENTRY LOCK. Compare text for description.

### Experimental Protocols

Experiment A: Correlation between hematological and pulmonary changes in normal rats. Ten normal rats were placed into the chamber. At 12, 24, 36, 48 and 60 hours two animals were removed into the tent, anesthetized and prepared for blood and lung study as mentioned above. Simultaneously two previously matched control animals were treated the same way.

Experiment B: Effect of  $\alpha$ -tocopherol deficiency on hematological and pulmonary changes induced by oxygen. Of 20 related rats, 4 weeks old at the beginning, 12 were fed an  $\alpha$ -tocopherol (vitamin E)-free diet (NAFAG, Gossau, Switzerland), the others being fed on standard rat chow of the same producer. After three weeks, 9 tocopherol-deficient rats and the 8 controls were placed into the chamber in 98% O<sub>2</sub> at 760 mm Hg. The animals were removed from the chamber and prepared according to the above-mentioned procedure according to table I. Three tocopherol-deficient rats were kept in room air as controls and processed at the end of the exposure experiment.



TABLE 1  
 PROTOCOL FOR EXPERIMENT B

Test Group:	Time of Removal from O <sub>2</sub> Chamber	Number of Animals	
		Tocopherol Deficient	Standard Chow
II	24 hours	2	2
III	36 hours	4	3
IV	48 hours	3	3
I	room air	3	—

## RESULTS

### Preliminary Experiments

A series of preliminary experiments were performed with normal rats in order to test the conditions of exposure and the time sequence of pulmonary changes. These established that in our presently available rat strain and under the above-mentioned exposure conditions the previously observed pulmonary changes (Kistler, Caldwell and Weibel, 1967) could be exactly duplicated, as well with respect to thickening of the barrier and primary damage of endothelial cells, as with respect to the time course, the animals rarely surviving for more than 72 hours in the chamber.

### Experiment A: Correlation between hematological and pulmonary changes in normal rats exposed to pure oxygen.

#### Pulmonary Changes

Figures 2 to 5 show the typical fine structural changes observed in these lungs. Very distinct alterations were seen at 48 hours where the interstitial space was widened by edema fluid accumulation (figure 3); the endothelial cells were in part structurally damaged, whereas the alveolar epithelium was mostly unchanged. At 60 hours (figure 4) the endothelial cells were heavily damaged and partly destroyed. The alveolar epithelium appeared altered, though less than the endothelial cells, and the alveoli were partially filled with edema containing fibrin threads.

The alveolar and interstitial edema occurring after 60 hours of oxygen breathing were particularly well demonstrated in a lung fixed by vascular perfusion according to the technique of Weibel and Gil (1968), as shown in figure 5.

At 36 hours some of these changes were already discernible, in particular interstitial edema, though less marked than at later time points. The lungs of animals exposed to O<sub>2</sub> for 12 and 24 hours did not differ from the controls.

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Key to symbols on electron micrographs

A	Alveolus	IN	Interstitium
C	Capillary	EP	Alveolar epithelial cell
EC	Erythrocyte	FB	Fibroblast
ED	Interstitial edema	FN	Fibrin
EN	Capillary endothelial cell	LC	Leucocyte

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Figure 2. ALVEOLAR CAPILLARY OF NORMAL RAT CONTAINING ONE ERYTHROCYTE (EC) AND ONE RETICULOCYTE (RC). x 16'800.



Figure 3. EDEMATOUS ENLARGEMENT OF INTERSTITIUM AFTER 48 HOURS OF OXYGEN. Epithelial cell not modified, some suggestion of endothelial cell damage (arrow) x 21'800.



Figure 4. AFTER 60 HOURS OF OXYGEN BREATHING THERE IS INTERSTITIAL EDEMA AND EXUDATION INTO ALVEOLI WITH DENSE PRECIPITATES (DP) AND FIBRIN THREADS (FN). x 13'250.



Figure 5. LOW POWER ELECTRON MICROGRAPH OF RAT LUNG EXPOSED TO OXYGEN FOR 60 HOURS FIXED BY VASCULAR PERFUSION. Interstitial edema (ED) and alveolar exudate (AE) are particularly well demonstrated. x 5'800.

In order to obtain an objective judgement of the ultrastructural changes the sets of electron micrographs derived from these preparations were judged in a double blind test by three independent experienced investigators. An arbitrary system of scores was used. Figure 6 summarizes the main results of this analysis; for epithelium and endothelium the quality of structural preservation was judged, for interstitium the degree of edematous swelling. It is clearly seen that the fine structural appearance of endothelial and epithelial cells deteriorates after 24 hours and that edema is recognized from 36 hours onwards.

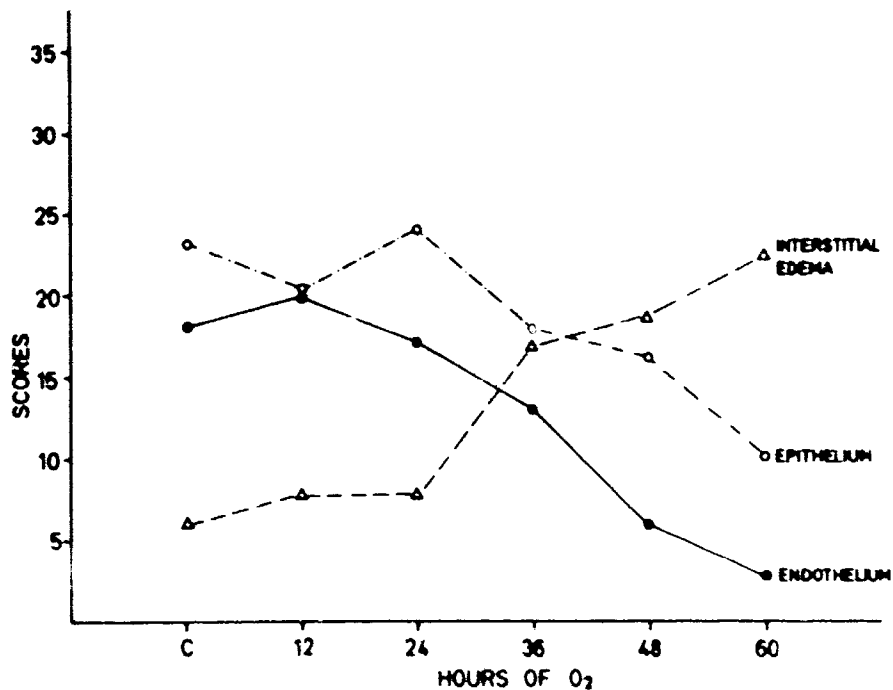


Figure 6. DOUBLE BLIND EVALUATION BY SCORE SYSTEM OF QUALITY OF EPI-  
THELIAL AND ENDOTHELIAL CELL PRESERVATION, AND QUANTITY  
OF INTERSTITIAL EDEMA.

As these findings were in keeping with our previous results, and as, furthermore, the hematological analysis did not reveal progressive changes with time, no morpho-  
metric measurements were done on these preparations.

### Hematological Changes

As figure 7 reveals, hematocrit and hemoglobin content of whole blood increased in the test animals with 48 and 60 hours of exposure to oxygen, whereas they were in the normal range (hemoglobin 14.6 bis 16 g%, hematocrit 43.5 Vol. %, Hagemann E. 1960), in the controls and in the test groups up to 36 hours. No significant changes could be seen in plasma hemoglobin; but the variation in these data was so large that a possible increase could well have been obscured by methodical error.

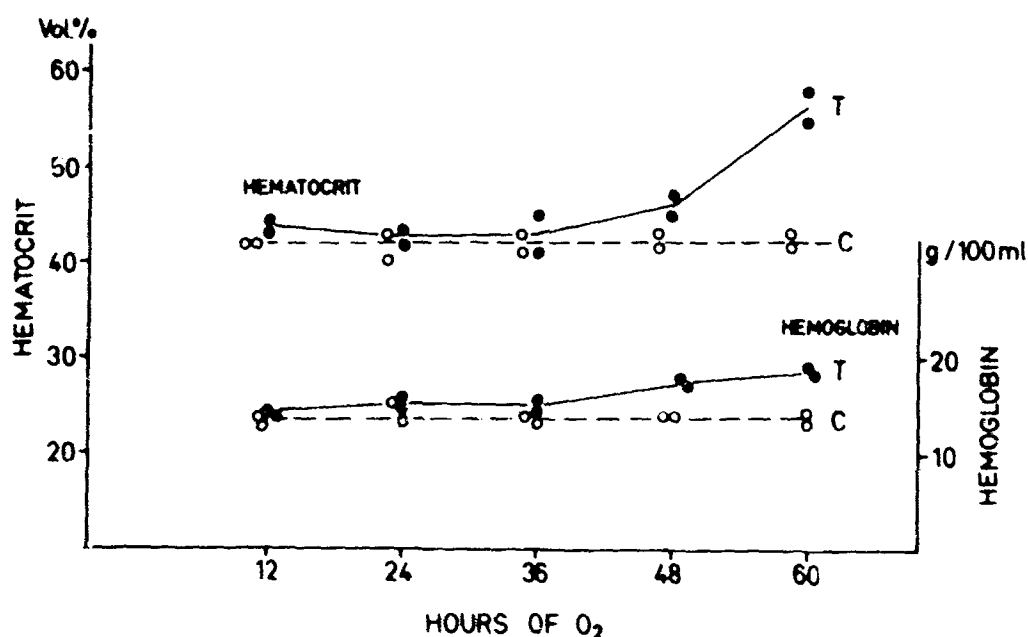


Figure 7. CHANGES IN HEMATOCRIT AND HEMOGLOBIN IN ANIMALS EXPOSED TO OXYGEN (FULL CIRCLES) AS COMPARED TO MATCHED CONTROLS KEPT IN ROOM AIR (OPEN CIRCLES).

The degree of hemolysis occurring in decreasing concentrations of NaCl is plotted in figure 8; the shaded area corresponds to the range of the control animals kept in room air, the individual curves to the exposed rats. It is noted that the curves of exposed animals are all essentially within the range of controls but that they appear shifted to the left-hand part.



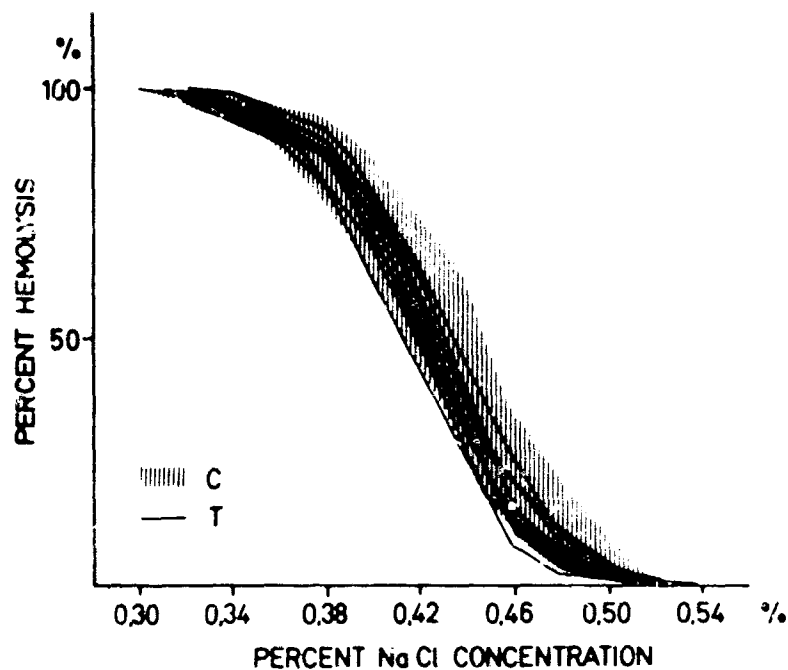


Figure 8. EFFECT OF OXYGEN BREATHING ON OSMOTIC FRAGILITY OF ERYTHROCYTES. Shaded area indicates range of controls, individual curves test animals.

This slight shift is significant at the  $P < 0.1$  level, as revealed by the comparison of the NaCl concentrations at which 50% hemolysis occurs (figure 9). A comparison of individual curves at each time point showed that the hemolysis curves had a clear tendency to shift to the left, i.e. to lower salt concentrations. This indicates that prolonged exposure to pure oxygen has slightly reduced osmotic fragility of red cells. It is of further interest that an apparent increase of immature red cells, particularly of reticulocytes was noted in the alveolar capillaries of the experimental animals.

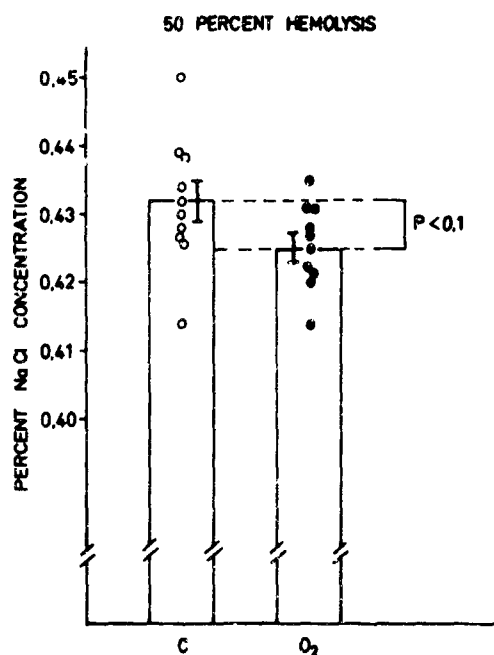


Figure 9. SALT CONCENTRATIONS AT WHICH 50% HEMOLYSIS HAS OCCURRED FOR CONTROLS (C) AND O<sub>2</sub> EXPOSED ANIMALS.

Experiment B: Effect of  $\alpha$ -tocopherol deficiency on hematological and pulmonary changes induced by oxygen

General Observations

All animals fed on  $\alpha$ -tocopherol deficient diet showed marked signs of developmental retardation: they are smaller and their weights were 25 to 60 percent below their matched controls (table II). One of the animals died spontaneously after two weeks.

Upon exposure to pure oxygen the health of the tocopherol-deficient animals deteriorated much more rapidly than that of controls: already after 24 hours they became cyanotic and lethargic, and one animal died after 36 hours, whereas it takes at least 48 to 60 hours for normal rats to develop clinical signs of respiratory difficulties.

TABLE II  
EXPERIMENT B: SYNOPSIS OF MORPHOMETRIC DATA

Diet	Hr	B.W.	Lung Volume	Vol. $V_c$	Alv. $S_A$	Cap. $S_C$	Barrier $\bar{T}$	Epith. $\bar{T}_{ep}$	Endoth. $\bar{T}_{en}$	Interst. $\bar{T}_{in}$	Harmonic Mean Barrier Thickness $\bar{T}_h$
	$O_2$	gr.	ml	ml	$m^2$	$m^2$	$\mu$	$\mu$	$\mu$	$\mu$	$\mu$
Tocopherol Deficient	0	140	6.30	0.58	0.46	0.43	1.02	0.25	0.35	0.42	0.43
		120	5.30	0.48	0.43	0.34	1.11	0.29	0.35	0.46	0.40
Tocopherol Deficient	24	105	5.85	0.56	0.47	0.37	1.62	0.37	0.35	0.89	0.45
		110	5.67	0.41	0.50	0.39	1.44	0.35	0.36	0.74	0.43
Standard	24	265	7.20	0.72	0.46	0.42	1.09	0.25	0.36	0.47	0.42
		265	7.47	0.82	0.46	0.41	1.29	0.35	0.43	0.51	0.41
Tocopherol Deficient	36	160	7.00	0.65	0.45	0.38	1.91	0.50	0.54	0.86	0.50
		155	6.30	0.62	0.54	0.46	1.40	0.32	0.36	0.73	0.45
Standard	36	210	7.30	0.82	0.62	0.52	1.20	0.30	0.39	0.49	0.43
		200	7.29	0.71	0.50	0.44	1.19	0.34	0.37	0.47	0.37
Tocopherol Deficient	48	155	6.30	0.42	0.48	0.33	2.06	0.41	0.53	1.10	0.65
		120	5.49	0.31	0.48	0.32	1.85	0.34	0.39	1.12	0.50
Standard	48	250	7.56	0.71	0.60	0.55	1.55	0.38	0.31	0.85	0.42
		280	7.47	0.77	0.54	0.40	1.80	0.41	0.40	0.99	0.40

### Pulmonary Changes

The animals fed on tocopherol-deficient diets which were kept in room air for the duration of the experiment showed a practically normal fine structure of the alveolar capillaries and of pulmonary tissue (figure 10), with the exception of the focal occurrence of cytoplasmic "ballooning" of epithelial cells as it is seen on figure 15 in an extreme case. Exposure to oxygen for up to 48 hours of the control rats fed standard chow led to the fine structural changes observed in experiment A with clearly recognizable change, particularly development of interstitial edema, occurring at 48 hours.

In contrast, the tocopherol-deficient rats developed severe changes already after 24 and 36 hours (figures 11, 12); these were comparable to the alterations observed in normal rats after at least 48 hours of exposure. The endothelium did still not exhibit marked structural damage, but the interstitium was edematous. At 48 hours, however, the endothelial cells appeared largely destroyed. Figure 13 shows a capillary with a collapsed lumen, swollen rough-surfaced endoplasmic reticulum cisternae, and myelin figures in a mitochondrion. Further examples of capillary closure with endothelial destruction appear in figures 14 and 15. In figure 14 the outline of the cells is barely recognizable; the perinuclear cisterna is greatly swollen.



Figure 10. ALVEOLAR CAPILLARY OF TOCOPHEROL DEFICIENT RAT KEPT IN ROOM AIR AS CONTROL. x 11'000.



Figure 11. ALVEOLAR CAPILLARY OF TOCOPHEROL DEFICIENT RAT EXPOSED TO  $O_2$  FOR 24 HOURS. Note partial destruction of capillary endothelium and formation of interstitial edema.  $\times 15'000$ .

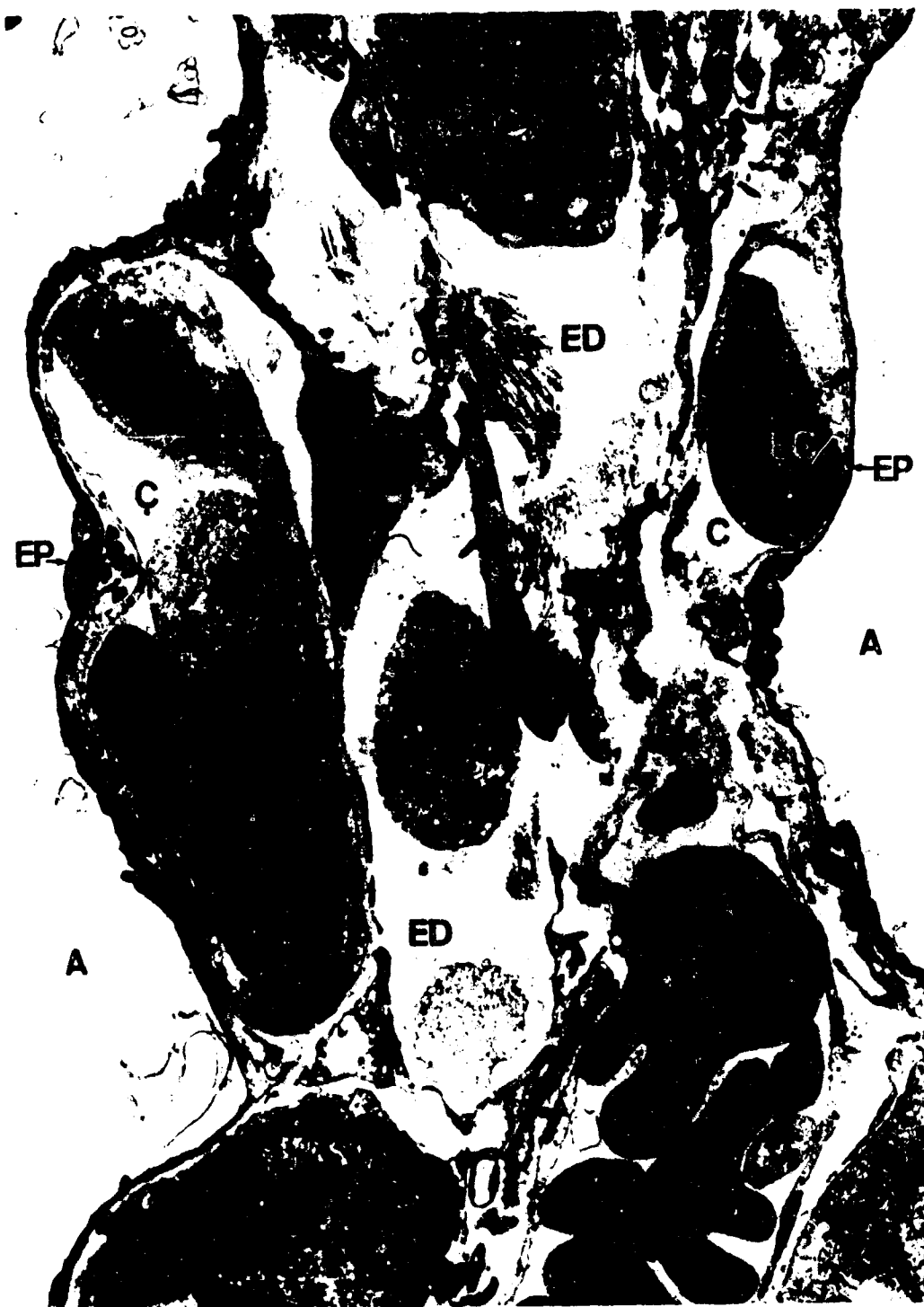


Figure 12. INTERALVEOLAR SEPTUM OF TOCOPHEROL DEFICIENT RAT EXPOSED TO  $O_2$  FOR 36 HOURS. Edematous enlargement of interstitium with some macrophages (MP) x 10'300.



Figure 13. TOCOPHEROL DEFICIENT RAT, 48 HOURS OF  $O_2$ . Endothelium of capillary with collapsed lumen (arrow) shows greatly swollen endoplasmic reticulum cisternae (ER) x 31'200.



Figure 14. TOCOPHEROL DEFICIENT RAT 48 HOURS OF  $O_2$ . Destroyed capillary; endothelial cell partly disrupted and partly swollen. Enlargement of endoplasmic reticulum and perinuclear cisterna (PC). Epithelial cells structurally well preserved. x 21'800.





Figure 15. INTERALVEOLAR SEPTUM OF TOCOPHEROL DEFICIENT RAT EXPOSED TO OXYGEN FOR 48 HOURS. Complete closure of capillaries due to swelling and disruption of endothelial cells. Epithelial cells show two examples of cytoplasmic "ballooning" which is also observed in controls; otherwise the epithelium appears well preserved. x 21'800.

The alveolar epithelium appeared unchanged over vast areas; however in some regions it exhibited "blebs" of swollen cytoplasm extruding towards the alveolar space (figure 15); but such changes could also be observed in the tocopherol deficient rats kept in room air, as mentioned previously.

The morphometric findings are summarized in table II. The most striking change relates to the thickness of the air-blood barrier (figure 16). The progressive increase in barrier thickness in the animals fed standard diet is in keeping with the previous findings: the interstitium doubles its size after 48 hours of oxygen, whereas endothelial and epithelial thickness remain unchanged. In the tocopherol-deficient animals the interstitium has more than doubled its volume after 24 hours and continues to increase. From 36 hours on endothelium and epithelium appear enlarged, which goes along with their observed cytoplasmic swelling (figures 12 to 14). The effect of capillary destruction becomes apparent after 48 hours in a reduction of the capillary volume (figure 17). In normal rats this occurred after 72 hours only.

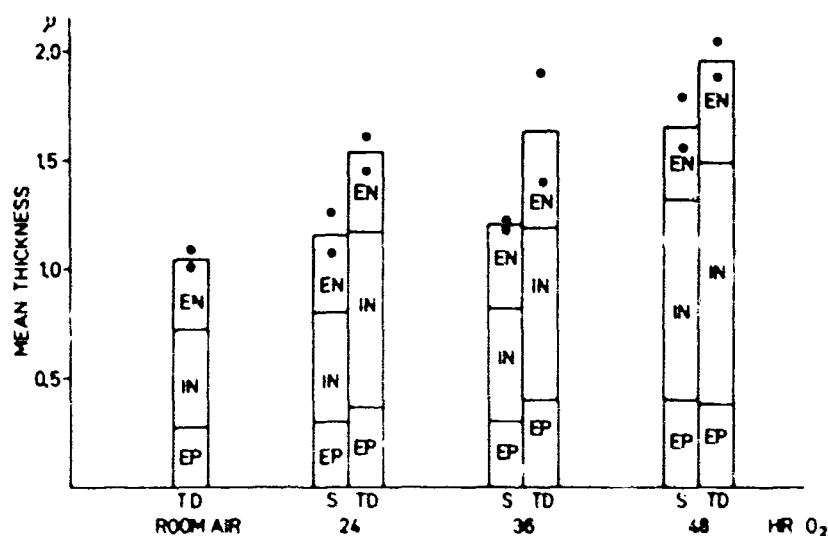


Figure 16. CHANGE IN THICKNESS OF AIR-BLOOD BARRIER AND ITS COMPOSITION IN TERMS OF ENDOTHELIUM (EN), INTERSTITIUM (IN), AND EPITHELIUM (EP) IN TOCOPHEROL DEFICIENT RATS (TD) AS COMPARED TO NORMAL RATS FED STANDARD DIET (S).

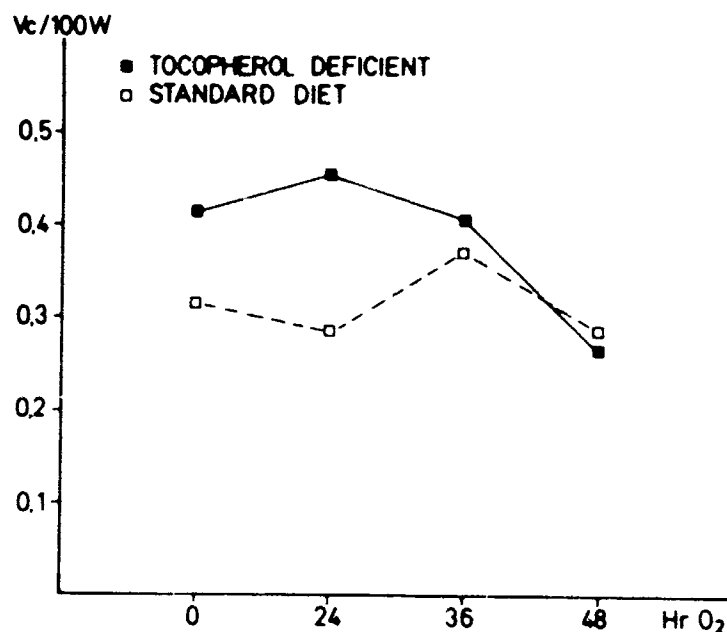
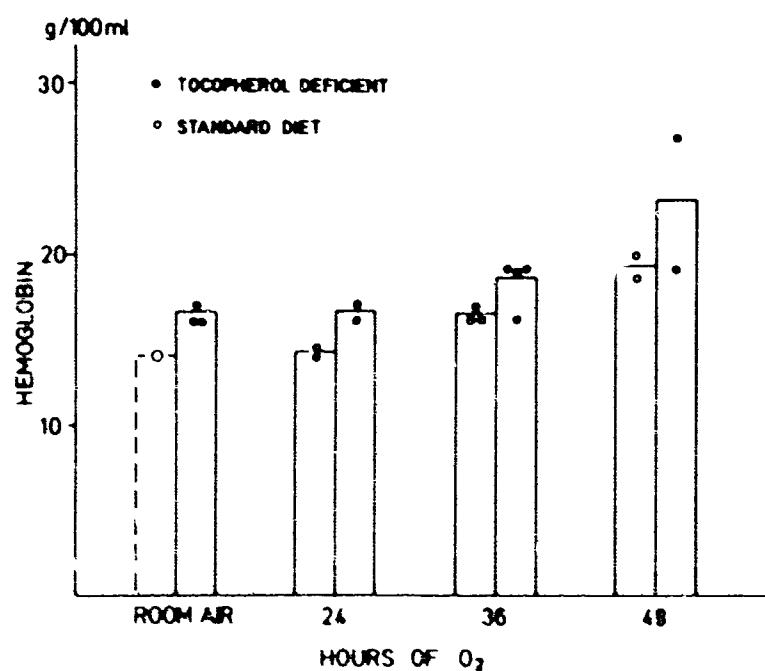
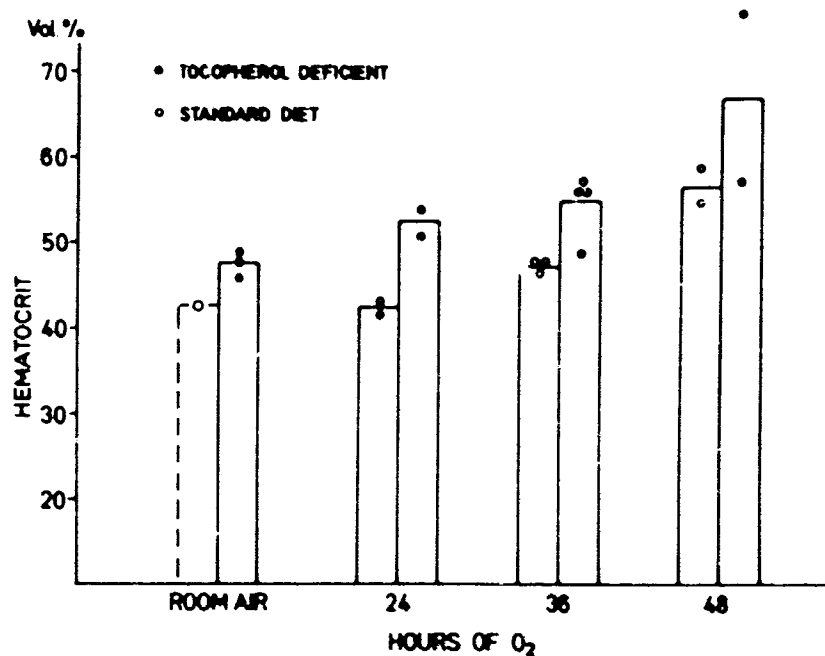


Figure 17. CAPILLARY VOLUME IN TOCOPHEROL DEFICIENT (FULL SYMBOLS), AND STANDARD DIET RATS (OPEN SYMBOLS), IN ROOM AIR AND DURING EXPOSURE FOR 24, 36 AND 48 HOURS TO OXYGEN.

#### Hematological Changes

Hematocrit and whole blood hemoglobin were increased in the tocopherol-deficient rats exposed to oxygen, as well with respect to room air controls as to exposed normal rats (figures 18 and 19).

The osmotic hemolysis curves, presented in figure 20, showed a most interesting pattern: whereas the room air controls of tocopherol-deficient rats had their curves shifted towards lower NaCl concentrations, as compared to normal rats exposed to oxygen, suggesting reduced osmotic fragility, the oxygen exposed tocopherol-deficient rats showed a rapid and marked shift to higher concentrations. This must be interpreted as rapid and progressive increase in osmotic fragility.



Figures 18 and 19. CHANGE IN HEMATOCRIT AND HEMOGLOBIN IN TOCOPHEROL DEFICIENT (FULL DOTS), AND STANDARD DIET RATS (OPEN CIRCLES) IN ROOM AIR AND DURING EXPOSURE TO OXYGEN.

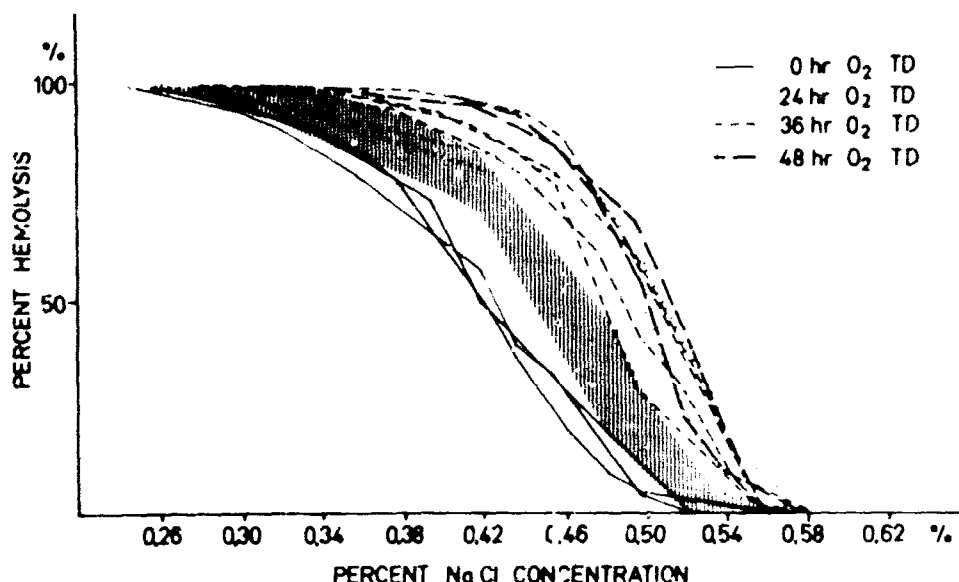


Figure 20. EFFECT OF OXYGEN BREATHING ON OSMOTIC FRAGILITY OF ERYTHROCYTES IN TOCOPHEROL DEFICIENT RATS (TD). Shaded area indicates range of hemolysis curves in normal rats exposed to oxygen for 24 to 48 hours.

This is further documented in figure 21 where the concentrations at which 50% hemolysis occurred are plotted against exposure time. That there is a progressive change in osmotic fragility is clearly revealed by the change in shape of the upper half of the curves (figure 20), particularly in the region of 80 to 90% hemolysis, which gradually shifts from 0.33 to 0.44 percent NaCl at 90% hemolysis (figure 22).

Electron microscopy revealed very bizarre shapes of erythrocytes to develop after 24 to 36 hours (figure 23). The membrane appeared to become invaginated; this resulted in the picture of large membrane bound vacuoles within the cells. It may however well be that these vacuoles are also deep invaginations of the surface membrane cut transversely by the section.

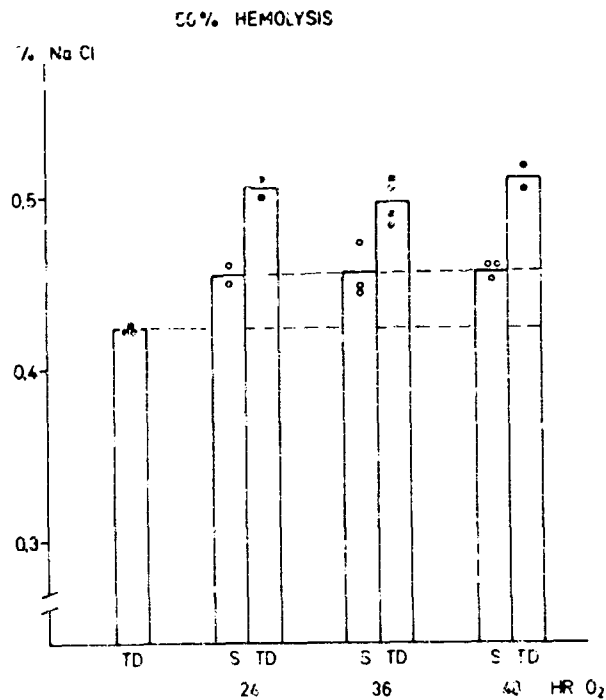


Figure 21. SALT CONCENTRATIONS AT WHICH 50% HEMOLYSIS HAS OCCURRED FOR TOCOPHEROL DEFICIENT (FULL DOTS) AND NORMAL RATS (OPEN CIRCLES) EXPOSED TO OXYGEN.

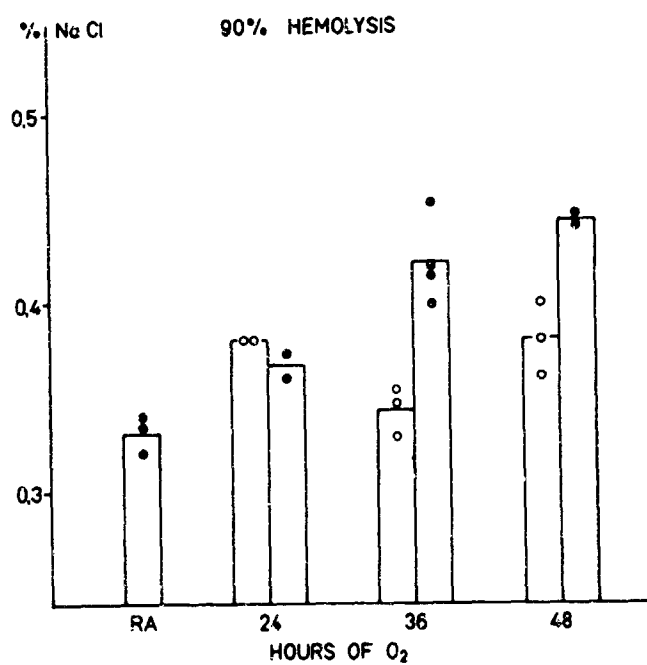


Figure 22. SALT CONCENTRATIONS AT WHICH 90% HEMOLYSIS HAS OCCURRED FOR TOCOPHEROL DEFICIENT (FULL DOTS) AND NORMAL RATS (OPEN CIRCLES) EXPOSED TO OXYGEN.



Figure 23 (a).





Figures 23. STRUCTURAL CHANGES IN ERYTHROCYTES OF TOCOPHEROL DEFICIENT RATS EXPOSED TO OXYGEN FOR 24 (a) AND 36 (b) HOURS CONSIST IN INVAGINATIONS OF MEMBRANE (ARROW) WITH CONSEQUENT VACUOLIZATION AND DISTORTION OF SHAPE. a: x 45'500  
b: x 64'500.

## DISCUSSION

### 1. Hematological Changes Induced By Oxygen

It was most interesting to note that exposure to oxygen at 760 mm Hg led to a slight reduction in osmotic fragility of erythrocytes. This result was not highly significant ( $P < 0.1$ ), mainly because the osmotic hemolysis curves from exposed animals were all within the range of parallel controls, but the trend is still evident. At first sight, this finding is in apparent contradiction to the observation by Danon (1966) that osmotic fragility of  $O_2$ -exposed rabbits is increased. But this may also mean that, in the interpretation of Danon, "old" cells become rapidly damaged by oxygen which leads to their elimination from the blood within a few hours. The remaining population of erythrocytes would then be relatively "young" which is expressed in a reduced osmotic fragility.

The progressive increase in osmotic fragility of erythrocytes of  $\alpha$ -tocopherol deficient rats is in line with the observations of other authors, particularly the group of Mengel (Mengel, 1964; Kann, 1969). Whereas most of the work has been done with  $O_2$  applied at several atmospheres of pressure, in a recent article Goldstein and Mengel (1969) have shown that hemolysis occurs in mice also at lower pressures though after protracted periods of time. Extrapolating to 1 atm. their curve on the dependence between pressure and time for causing hemolysis in tocopherol-deficient mice, grossly detectable in vivo hemolysis should occur after some 75 hours of exposure. It is therefore quite likely that an increase of in vitro osmotic fragility precedes overt hemolysis.

We were not able to detect hemoglobinemia as clear signs of in vivo hemolysis. This does not mean that no hemolysis has occurred, as the method used to determine plasma hemoglobin showed very large variations even in controls, which made an interpretation of the data impossible.

That a replacement of red cells must take place was evidenced by the observation of a marked increase in immature red cells, mainly reticulocytes, in the alveolar capillaries.

One intriguing finding was the increase in hematocrit and total hemoglobin, which was observed in both experimental groups to occur in the later phase of  $O_2$  poisoning. This may be explained by two alternative processes: either it is the expression of hemoconcentration due to fluid loss from the blood in connection with the formation of edema, or the observed increase in reticulocytes must be interpreted not as replacement of destroyed and eliminated red cells, but as supplemental release of new erythrocytes into the blood. The present experiments would tend to favor the first hypothesis: it can be easily calculated that the observed accumulation of fluid in pulmonary interstitium and alveoli, and in the pleural cavity can amount to something of the order of 2.5 ml or more. This would be nearly sufficient to raise the hematocrit of a 200 g rat from 42.5 to 56%, as observed in experiment A at 60 hours of exposure. The contribution of an elevated number of reticulocytes to this increased hematocrit is furthermore unlikely because total hemoglobin increases in parallel and reticulocytes are known to contain less hemoglobin per unit cell volume than mature cells.

## 2. Pulmonary Changes

The general pattern and sequence of cytotoxic oxygen effect on the lung observed in both experiments agrees with our previous observations on rat lungs (Kistler, Caldwell and Weibel, 1967) and also with the initial changes in the monkey lung (Kapanci, Weibel, Kaplan and Robinson, 1969). In the normal rats we observed a delay of 24 to 36 hours before the onset of clearly discernible morphological changes. In the tocopherol-deficient animals however the cellular damage occurs within 24 hours, and at that time it corresponds in degree about to the changes observed after 48 hours of oxygen breathing in normal rats. At 48 hours capillary destruction is at least as intense as that found after 72 hours in normal rats.

The alveolar epithelium of tocopherol deficient rats appears rather well preserved throughout the experiment, except for some cytoplasmic "ballooning" in thin portions of squamous cells. Two examples of this change are shown in figures 13 and 15. However, these changes were not frequent, and they could even be observed in lungs of tocopherol-deficient rats kept in room air. It can thus not be excluded that this is a change induced by tocopherol deficiency, which may perhaps become aggravated in a high  $O_2$  atmosphere.

### 3. Correlation of Hematological and Pulmonary Changes

The exposure of normal rats to one atmosphere of pure oxygen could not reveal any evidence on a temporal or causal correlation between red cell and pulmonary damage, mainly because no progression in the red cell changes could be observed. Nevertheless such a correlation could not be excluded, since it may well be argued that the apparent reduction of osmotic fragility of red cells is the cause for the time lag in the occurrence of cytotoxic effects.

This argument may be particularly opportune in the light of the findings on tocopherol-deficient rats where we did not only find an aggravation but also a precipitation of cytotoxic effects. The onset of cytotoxic effect of oxygen occurred within the first 24 hours and paralleled a rapid increase in osmotic fragility of erythrocytes. This indicates a temporal colinearity in the cytotoxic effects on red cells and endothelial cells of alveolar capillaries.

Further experiments will be needed to show whether there is a causal interrelationship between red cell and lung damage. It can, for example, not be excluded that tocopherol deficiency acts also directly on the pulmonary cells in the sense of increasing their susceptibility to high oxygen tensions. But we then are still left with the problem why alveolar epithelial cells have not become more susceptible to oxygen as they are remarkably well preserved even after 48 hours of oxygen while the endothelial cells have undergone very extensive destruction.

### 4. Conclusions

This experiment has shown that pulmonary damage due to high oxygen tensions becomes more severe and develops at an earlier time point in tocopherol deficient rats, and that this is paralleled, or maybe preceded, by the well-known increase in osmotic fragility of erythrocytes. To prove a causal relationship between hematological and pulmonary changes further experiments, using in part the model of tocopherol deficient animals, will be required.

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## DISCUSSION

DR. CAMPBELL: How much of the lung did you prepare for electron microscopy and how much did you review to come up with these nice demonstrable changes? In other words, can you process only a small sample of the lung and see these things?

DR. WEIBEL: Oh, yes. I can tell you that actually the quantity of the material that we have really quantitatively analyzed for morphometry is zero because we are using point sampling techniques. I don't think I can go into the details of the sampling problem in morphometry. I think we have gone into that on previous occasions, but essentially the main question is not how much tissue you examine for diffuse changes as we have them here, but how well you sample it from the lung and these are strictly random sampling techniques; I should perhaps qualify that--they are systematic sampling techniques which are unbiased. Also the measurement techniques are unbiased.

DR. LEON: I would just like to say that on your osmotic fragilities, we are working with Dr. Danon's Fragilograph and of course the one drawback to the fragilograph, which Dr. Danon recognizes, is that the scale is condensed at that end that we are most interested in; that is at the end where the older cells start to hemolyze first, and we are developing a computer program that will take the fragilograph recording, convert it and linearize it, stretch it out, and will then quantitate the red blood cells in each molarity or percentage salt concentration, and give you a quantitation. We don't quite have it developed yet, but if anybody is interested in the program, just write me a letter and I'll send it to him just as soon as we get it finished.

DR. WEIBEL: I could perhaps comment here that the data you have seen here has not been done with a fragilograph for only one reason. That is the delivery time was a little over nine months and in the meantime we wanted to get our experiments started, so we did it the hard way by using little tubes and pipetting the different salt concentrations; however this may account a little bit for the spread of the curves, but we now have repeated the studies by using the fragilograph and we come up with the same results.

DR. CAMPBELL: This morning there seemed to be some question that the changes in the red cells as the result of the oxygen exposure might be not actually intravascular hemolysis but might be an effect which is causing them to be removed in the RE system. Yet you suggest that these changes are occurring intravascularly to the point of actual hemolysis, and I think in one slide you showed morphologic evidence that this is true. Is this a difference in degree of the same type of change, where before there may not have been much in the way of intravascular hemolysis but the same sort of change in a lesser degree causing them to be recognized and removed by the RE system?

DR. WEIBEL: Well, I think that you may get a very small amount of leakage of hemoglobin or perhaps only ferrous ions from the interior of the red cells, when you get the peroxidative changes in the membrane. These are likely to take place right in the lung, depending on how fast this can happen, you may get the leakage there into the capillary whereby these iron ions could be picked up by the endothelial cells immediately, perhaps stuck to the extracellular coat, so that you couldn't pick them up in the venous blood after passing through the lung. You need only trace amounts for the in vitro causation of peroxidation of lipids or enzyme inhibition, only trace amounts of ferrous oxides. You can see just a little bit get out. As a matter of fact, I have shown you here pictures of damaged red cells. Now we have also found, and this has been published in the previous papers, mainly in the monkey, lung capillaries which had a plasma of about the same density as the interior of the red cells so that it appears that there was a profuse loss of the material that gives the red cells its density--mainly hemoglobin into the plasma.

We also found in the oxygen exposed animals erythrocytes of various different electron densities, some have a grey shade, some are almost black. This can be due to a swelling, taking up of a fluid, or to a partial loss of the capillary. Of course, this does not prove anything but circumstantial evidence.

MAJOR SINCLAIR: I believe you said the oxygen exposed cells shifted to the left indicating increased resistance to fragility. Can you explain that?

DR. WEIBEL: That is just a matter of how you set up your scale. On the left hand side are lower concentrations of sodium chloride. So if a cell hemolyzes at a lower concentration of sodium chloride, it will have a high resistance to osmotic shock or a reduced fragility, whatever you want to say.

DR. TOWNSEND: This has been extremely interesting. There are a number of papers in the last year or two dealing with hemolytic anemia, hemolytic processes, with tissue damage, but time does not permit us to mention these, but if anybody is interested, I have looked up some of the literature on this.

## ELECTRON MICROSCOPIC EVALUATION OF KIDNEY CHANGES IN TOXICITY STUDIES

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### INTRODUCTION

Studies previously reported at these meetings indicated various changes in the tubular cells of kidneys of animals exposed to pure oxygen, oxygen/nitrogen mixtures, and monomethylhydrazine. Electron microscopic studies were performed on serial renal biopsies from monkeys and dogs following translocation of the left kidney to a subcutaneous site to facilitate the biopsy procedure. Although cellular alterations varying with the type of exposure could be demonstrated for each group, these studies were deficient in lacking control animals exposed in Thomas domes at ambient atmospheres, and adequate control studies of the effect of the translocation procedure. The purpose of this presentation is to report the findings in these control animals, and to place the previously reported findings in proper perspective.

### MATERIALS AND METHODS

Monkeys and dogs were subjected to unilateral translocation of the kidney and then exposed in Thomas domes to one of the following:

- 1) 100% oxygen at 15 PSIA for 3 - 12 days
- 2) 100% oxygen at 5 PSIA for 3 - 7 months
- 3) 68% oxygen, 32% nitrogen at 5 PSIA for 3 - 7 months
- 4) Ambient atmospheres at 15 PSIA for 5 months

In addition, groups of animals were injected with various amounts of monomethylhydrazine (MMH) intraperitoneally (George, 1968).

Serial renal biopsies were performed on the translocated kidney before, during, and after exposure. Complete necropsies were performed within one year after the completion of each experiment. Methods of translocation, atmospheric exposure, biopsy, tissue processing and electron microscopic investigation were reported previously (Kaplan, 1967; Felig, 1965; Mautner, 1966).

## RESULTS

1) Morphologic changes due to translocation: It was previously reported that a number of animals showed changes in the translocated (as opposed to the *in situ*) kidney several months following the translocation procedure, irrespective of the type of exposure (Mautner, 1968). Further studies indicate that such alterations, far from being occasional complications of the translocation procedure, can be found in approximately 90% of translocated kidneys within 6 - 8 months following the procedure, provided only that a sufficient number of sections from each such kidney is examined. Although these changes varied in distribution and severity, they had the following features in common:

- a) Tubular cell degeneration, frequently to the point of necrosis, involving both proximal and distal tubules.
- b) Increase in interstitial connective tissue.
- c) Scar formation.

Occasionally, vascular changes were observed in medium-sized arteries.

The nature of the scars varied markedly. The most common type consisted of thin bands of fibrous tissue extending from the medulla to the cortex, usually perpendicular to the plane of the capsule. Others were thick, obviously replacing a number of adjacent degenerated nephrons. Usually intact or partially intact glomeruli were found within the scars. The degree of indentation of the capsule at the insertion of the fibrous bands varied, but in the majority of cases was slight and occasionally absent. Connective tissue stains were weakly positive for collagen. All these factors suggest that these lesions were of relatively recent origin.

In addition to fibrous bands, focal areas of fibrosis were scattered throughout these kidneys. These varied in size, shape, and distribution, but the majority were located in the cortex. In a few instances flat scars were found immediately below, and parallel to, the renal capsule. Focal thickening of the capsule was frequent.

There was substantial variation in the degree of inflammation found in the various scars. The inflammatory exudate was usually of a chronic nature, consisting primarily of lymphocytes and macrophages, but occasional polymorphonuclear leukocytes were found.

Electron microscopy of the scarred areas (figure 1) showed the expected increase in collagen fibers between degenerating tubules, mixed with fibroblasts, macrophages, and lymphocytes. The remaining identifiable tubular cells showed loss of cytoplasm, and the tubular basement membranes were markedly thickened.

Electron microscopy of the unscarred areas (figure 2) showed normal structure in unexposed and MMH exposed animals.



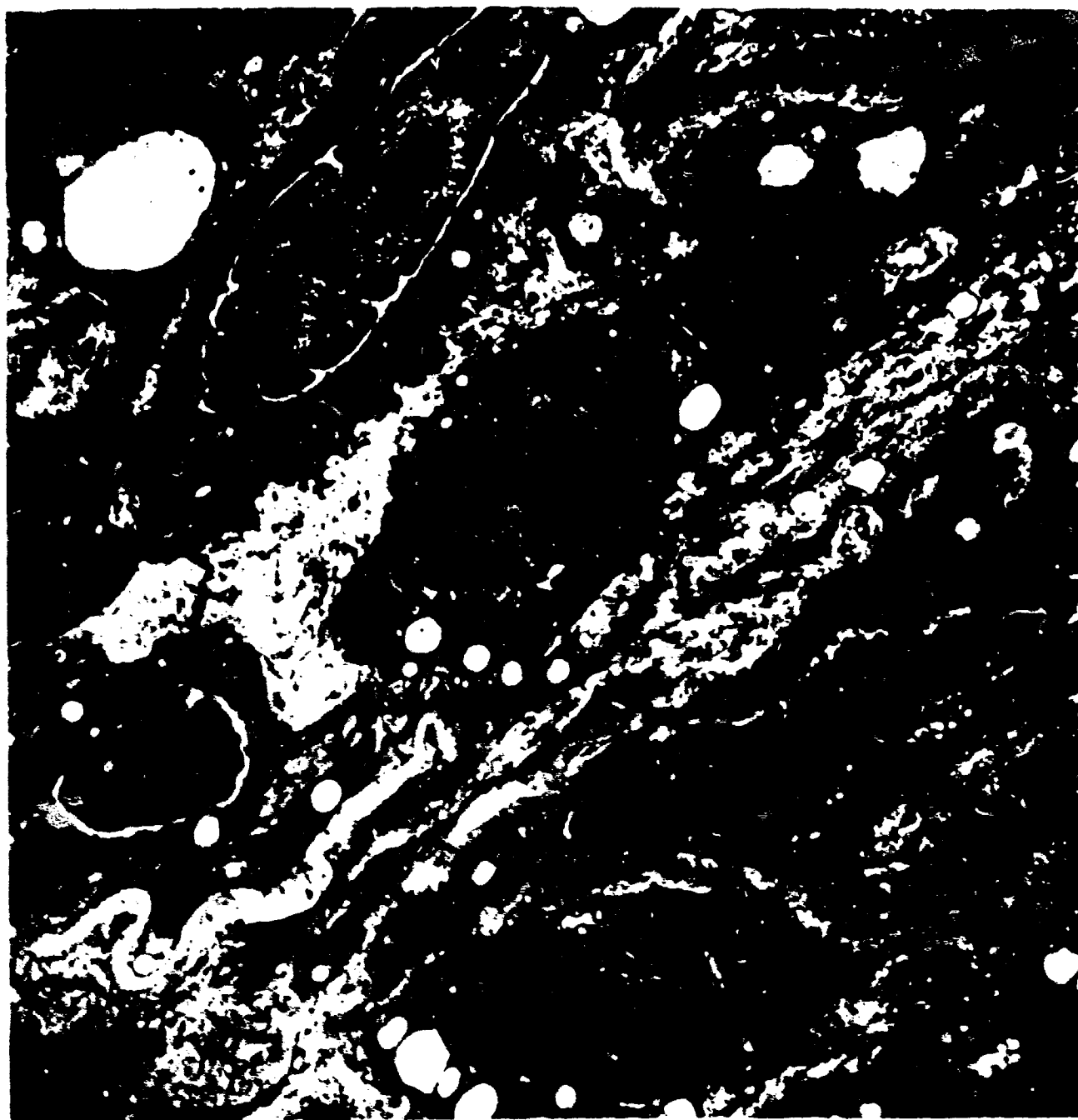


Figure 1. ELECTRON MICROGRAPH OF RENAL CORTEX OF MONKEY, 5 MONTHS AFTER TRANSLOCATION, 5 MONTHS AFTER EXPOSURE TO MMH. Scar containing fibroblasts and inflammatory cells in a loose matrix of collagen fibers, indicating recent damage. X10,900

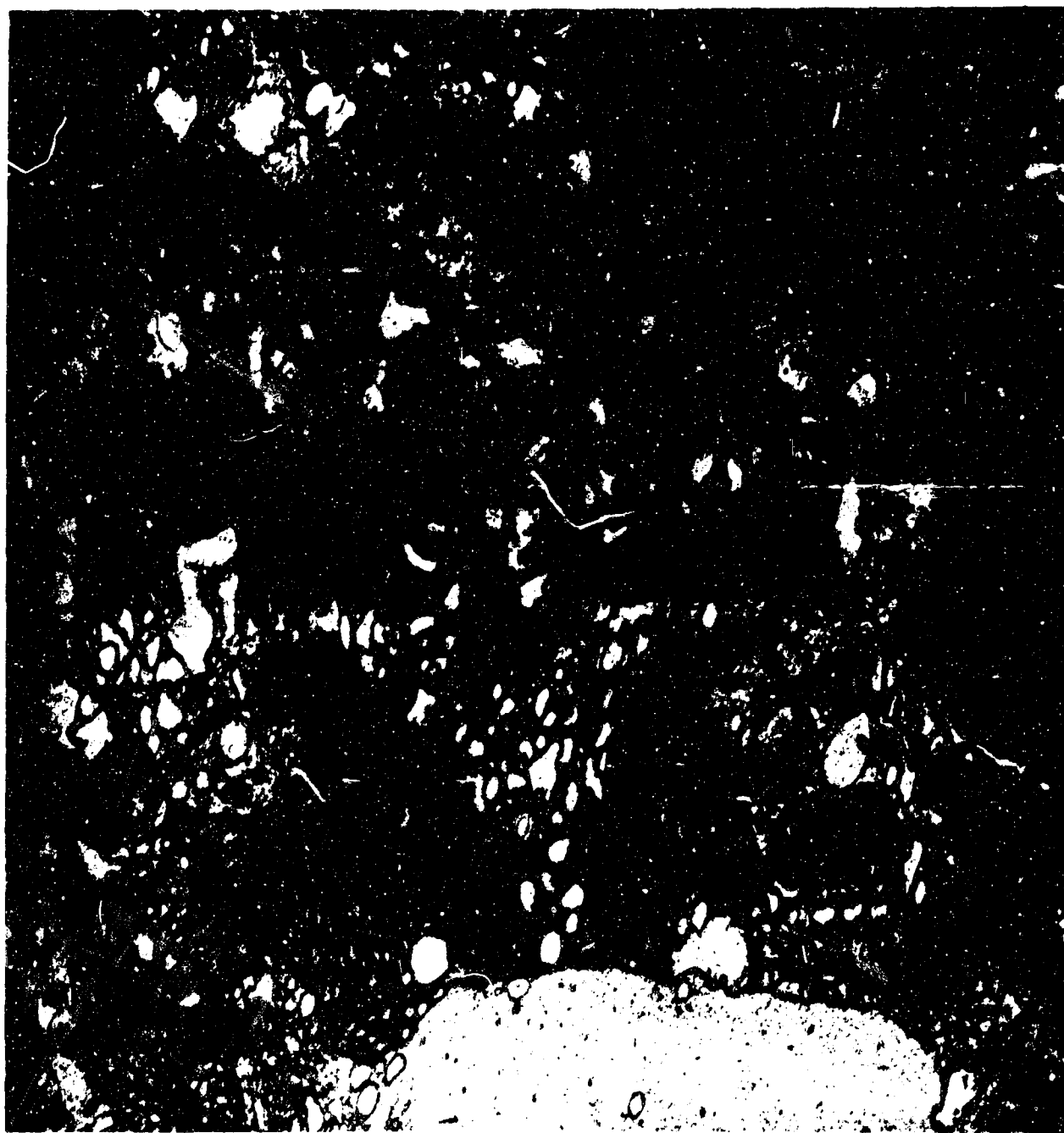


Figure 2. SAME AS FIGURE 1, UNSCARRED AREA. Proximal tubule with basement membrane at top, nucleus at right edge, brush border and lumen at bottom, showing normal architecture and cytologic details. X25,000

2) Atmospheric exposures: The proximal tubular alterations following exposure to pure oxygen, both at 5 and 15 PSIA, have been reported (Mautner, 1966, 1968). These consisted of marked irregularity in the profiles and cristae of the mitochondria of proximal tubular cells, and an increase in the number of microbodies. These changes were partially reversed upon continued exposure. Animals exposed to 68% oxygen, 32% nitrogen at 5 PSIA showed similar, though much less severe, mitochondrial alterations which were not reversible, and were not accompanied by an increase in microbodies. Animals which were exposed in the same way (Thomas dome) to ambient air at 15 PSIA (figure 3) showed exactly the same changes as those reported in the animals exposed to the oxygen-nitrogen mixture. There was smudging and disappearance of mitochondrial cristae and some degree of swelling of the mitochondria. The architecture of the tubules was preserved. There was no discernible increase in the number of microbodies.

3) Monomethylhydrazine exposure: George (1968) reported extensive vacuolization of proximal and distal tubular cells during the acute stage of MMH intoxication. Electron microscopic studies of these animals six to twelve months after exposure reveal areas of scarring (figure 1) as in all animals following transplantation, and perfectly normal areas (figure 2). Consequently, residual changes due to exposure cannot be detected, although the existence of focal alterations cannot be ruled out.

## DISCUSSION

Unilateral translocation of the kidney to a subcutaneous site has been used in a variety of acute experiments requiring serial renal biopsies. In the present series, requiring exposure for many months following translocation, fibrotic changes have been found which interfere substantially with the interpretation of the exposure effects. All data presented have indicated that these are scars of recent origin, with as yet little or no cicatrization; and that the tissue between scars is normal in architecture and cytologic detail. It must be assumed, however, that as these scars contract, pressure effects on the surrounding tissue will make this material useless for toxicologic studies. Although the exact temporal relationships have not yet been worked out, these studies suggest that the translocation procedure can be used without reservation for acute experiments; that it can be used for chronic experiments up to approximately one year provided the degenerative changes caused by the procedure are taken into account; and that it is useless for long term experiments.

The cause of scarring following translocation cannot be determined from these findings. The thin bands perpendicular to the capsule resemble vascular scarring; the subcapsular, flat scars suggest a traumatic etiology; scattered, irregularly shaped areas of fibrosis, sometimes with intense inflammation, are reminiscent of interstitial pyelonephritis. Most likely, all three contribute to the development of these lesions.

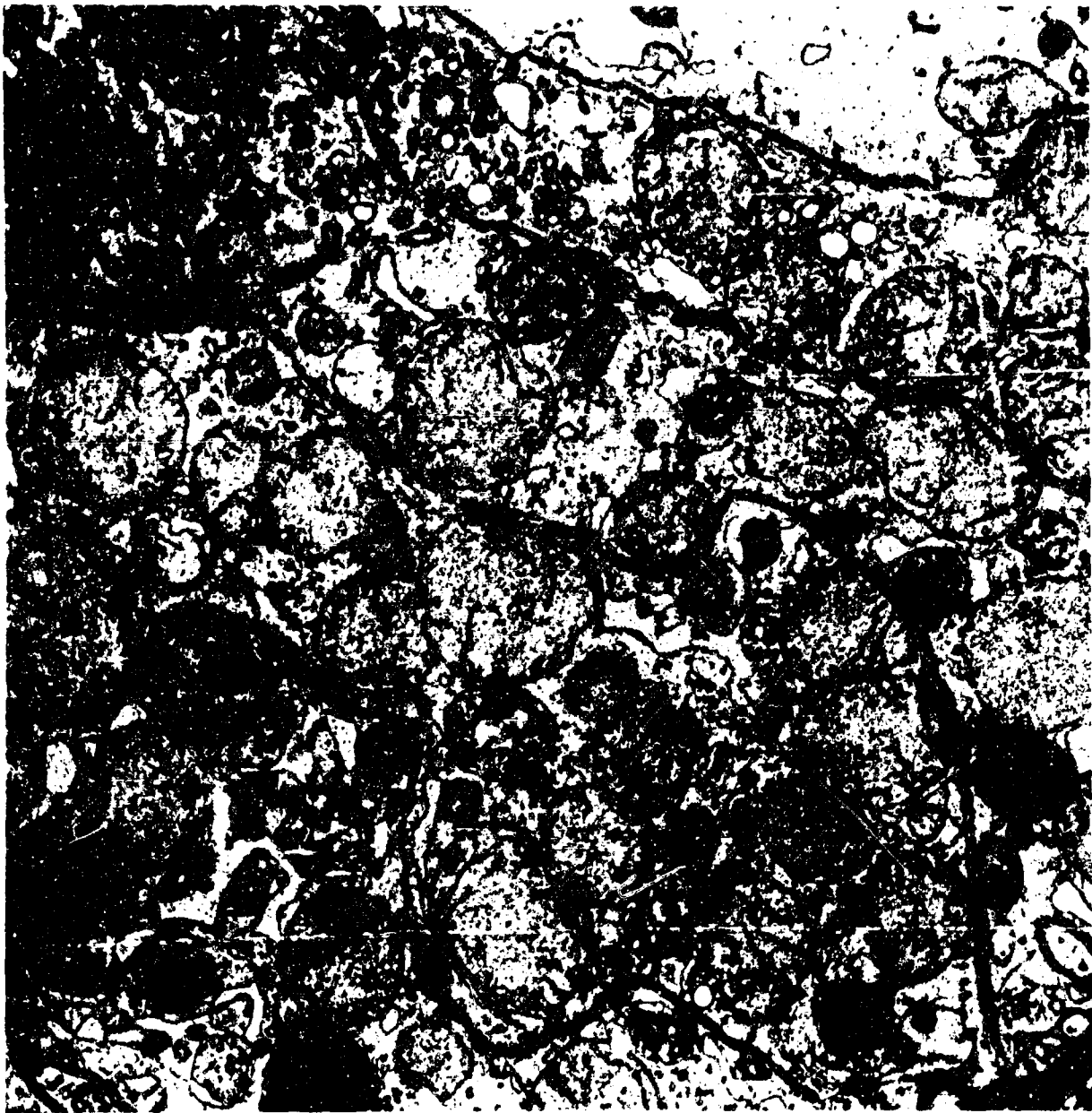


Figure 3. ELECTRON MICROGRAPH OF PROXIMAL TUBULE OF MONKEY EXPOSED TO AMBIENT AIR IN THOMAS DOME FOR 5 MONTHS. Brush border at upper left, basement membrane at lower right. Overall architecture is preserved, but mitochondria show irregular outlines, swelling, and loss of cristae. X22, 200

The finding of tubular alterations in animals exposed to ambient air in the Thomas domes suggests that there may be a substance within these domes causing these changes. There is no evidence to suggest that these changes are necessarily degenerative; they may be purely adaptive. It can only be concluded that animals exposed in Thomas domes are subject to some change in environment which causes slight submicroscopic alterations in the proximal tubules. Animals exposed to pure oxygen at either 5 or 15 PSIA show changes which are more severe and slightly different; animals exposed to 68% oxygen, 32% nitrogen, do not.

#### SUMMARY AND CONCLUSIONS

1) Translocation of the kidney for serial renal biopsy studies is a useful procedure for acute experiments, but causes degenerative changes which must be taken into account in chronic experiments and may make the procedure useless for long term experiments lasting more than one year.

2) Exposure of animals to ambient air in the Thomas dome causes slight submicroscopic alterations in the proximal tubular cells. It is not known whether these changes are degenerative or adaptive.

3) Animals exposed to pure oxygen show more severe changes than the control animals. Animals exposed to oxygen-nitrogen mixtures with a normal partial pressure of oxygen resemble those exposed to ambient air.

#### ACKNOWLEDGMENT

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## DISCUSSION

MAJOR CARTER: Did you ever observe any of the scarring in kidneys not biopsied? I'm curious about the scarring.

DR. MAUTNER: I didn't get the question.

QUESTION: Had all the exteriorized kidneys been biopsied at least once?

DR. MAUTNER: All were biopsied, yes, somewhere along the way. A couple were biopsied only terminally. Are you trying to suggest that the scars may be due to the biopsy?

MAJOR CARTER: Yes I am, because I did a few of those and my technique was very poor.

DR. MAUTNER: I would suggest that there may be as many as 36 of these scars in one kidney. Do you think you could get that many, even with a poor technique?

MAJOR CARTER: I don't know, poor as my technique was, there could have been. I think it might be worthwhile, if we are interested in using this any more, to take any number of animals and exteriorize them without biopsies and let you look at them again if you would be interested.

DR. MAUTNER: The animals that were killed yesterday--were they biopsied before?

MAJOR CARTER: Yes, they were.

MAJOR THEODORE: Could you discriminate between micro infarcts and secondary pyelonephritis on a long term? Because when you look at the renal artery you really don't have much room to play with it when you try to exteriorize a kidney. I'm just wondering how much of the changes, if you could tell (I know in chronic conditions if you were looking at scars it would be very difficult) but is there any evidence to suggest anywhere along the line ischemic infarctions then with superimposing pyelonephritis, which in a late stage you may not be able to tell?

DR. MAUTNER: I believe that there is an infectious component because some of the areas are so full of inflammatory material so that you can obviously get inflammation simply as a consequence of rephrosis, of cutting down the blood flow, and the question always is how much inflammation do you need to call it an infectious process

rather than simply a vascular process? The answer is I cannot distinguish with any degree of certainty. I just have a feeling that these animals are susceptible to various insults. That there is something that is done to the vasculature which causes at least a part of these scars and that there is an element of pyelonephritis or inflammation, no question about it.

MAJOR THEODORE: Just for my own benefit. I'm assuming that you are concluding that you can't really tell if there are any effects of oxygen toxicity on these studies.

DR. MAUTNER: Oh yes I can. I believe that if I stay out of the scar when I do electron microscopic work on these kidneys, I can make a distinction and there is some reason to support this because we did biopsies terminally on both sides of the explanted kidney and the one was not touched, you see, and the changes as far as the exposure changes that I am talking about are the same in the two kidneys.

MAJOR THEODORE: Right.

DR. MAUTNER: The changes are diffuse. The scars are focal and different from one kidney to the other.

MAJOR THEODORE: What about some of the vascular changes that you demonstrated? You had minimal thickening.

DR. MAUTNER: Very minimal.

MAJOR THEODORE: I'm just saying that a synergistic effect from two different processes may explain this. I'm not saying for sure, but I think caution is warranted.

MAJOR MAC KENZIE: Did you see similar lesions in the unexteriorized kidney?

DR. MAUTNER: No--Look I gave two talks here essentially. I talked about the exteriorization of the kidney and that was a unilateral phenomenon, and then I talked about changes due to exposures, that was a bilateral phenomenon.

DR. WEIBEL: How did you then sample the not exteriorized kidney?

DR. MAUTNER: We biopsied it at time of sacrifice.

DR. WEIBEL: I see.

DR. COULSTON: It is a beautiful piece of work, quite clear. Would you care to speculate on the mitochondrial regeneration or reappearance of mitochondria in these chronic studies?

DR. MAUTNER: I don't think that mitochondria really disappear and reappear. I think they are altered. I think that the cristae or internal structures sort of become vague or disappear actually in many of them.



DR. COULSTON: No generations of mitochondria involved there?

DR. MAUTNER: No evidence that there is and as far as speculating as to what the cause of this might be, I wouldn't want to say.

DR. COULSTON: I agree with your interpretation. The best way is to say it is a physiological adaptation to the particular chemicals that you are studying. You can't say that it is toxic or not toxic, but you can say that there is an alteration and this alteration is probably reflected in contrast with other things that are involved in the mitochondria. Did you see any changes in microsomes, in the microsomal area?

DR. MAUTNER: Well you know the kidney is not like the liver. It doesn't have the beautiful endoplasmic reticulum; surely it has some, although I did not see any changes.

DR. THOMAS: Dr. Mautner, did you say that the oxygen changes at 5 psi were reversible or showed a tendency to reverse?

DR. MAUTNER: Yes, the mixed gas and the dome control are not showing any change in reversibility.

AMRL-TR-69-130

SESSION IV

DETECTION AND CHARACTERIZATION OF CONTAMINANTS

Chairman

Mr. Milton L. Moberg  
Electronics Division  
Aerojet-General Corporation  
Azusa, California

## SOURCES AND REMOVAL OF CARBON MONOXIDE IN HYPERBARIC ATMOSPHERES

Merle E. Umstead, Ph.D.  
James K. Musick  
and  
J. Enoch Johnson, Ph.D.

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### INTRODUCTION

In closed atmospheres, carbon monoxide (CO) is a trace contaminant which merits special consideration because of its toxicity and ubiquity. In SEALAB II, the CO concentration gradually increased to about 30 ppm, and was strongly suspected of being the cause of headaches among the Aquanauts living under hyperbaric conditions (Saunders et al, 1967). The rate of buildup of CO in the habitat during the initial phase of the SEALAB Operation was about 1.4 liters per day, and its source was never established with certainty.

In experiments to 100 psig, it has been shown that the toxicity of CO to animals in high pressure helium-oxygen atmospheres is directly related to the partial pressure of the CO, at a constant partial pressure of oxygen (Siegel, 1969). During qualification dives to 600 ft for SEALAB III in a pressure chamber, atmospheric CO was monitored, and blood samples from some of the divers were analyzed for carboxyhemoglobin. The carboxyhemoglobin levels found correlated well with the partial pressure of CO in the atmosphere as predicted by the Haldane Equation. Since the physiological action of CO is a function of its partial pressure, problems associated with CO become accentuated in hyperbaric systems. For example, for the continuous exposure of men at a pressure of 20 atmospheres, the concentration of CO must be kept below one to two parts per million. Maintaining such low limits for this contaminant increases the problems of monitoring this gas and removing it from the atmosphere.

There are many potential sources of CO in hyperbaric atmospheres and undersea habitats. These include cooking, reactions of activated charcoal, outgassing of materials of construction, and the sea itself. Man himself produces about 10-20 cc of CO per day (Cooper, 1966). A laboratory study has been carried out on potential sources of CO, with emphasis on cooking and the reaction of oxygen with activated carbon. In order to accomplish these studies and also to monitor CO in field situations, it was necessary to develop sensitive methods of analysis for this gas.

## SOURCES OF CARBON MONOXIDE

### Cooking

The cooking of foods is a very likely source of CO in closed atmospheric environments, particularly if the food is somewhat burned. A study has been carried out to determine the volume of CO that might be produced by cooking both in air atmospheric pressure, and in a high pressure helium-oxygen atmosphere with a low oxygen concentration. As examples of food which seemed likely to produce CO when cooked, bread was toasted; and suet, representing fat, and steaks were broiled.

The cooking experiments were carried out in a pressure chamber which had a volume of 142 liters, a working pressure of 315 psia, and was equipped with a fan to ensure mixing of the gases. After filling the chamber with the desired atmosphere to the desired pressure, the food samples were cooked in it to various degrees of charring. Carbon monoxide concentrations in the chamber were measured before and after the cooking, and from the difference in these values, the amount of CO evolved from the sample was calculated.

The food was heated in a toaster (or broiler) made of stainless steel. It was a simple framework about 10 x 5 x 4 inches high, which supported an electrical heating plate at the top, a shallow pan at the bottom, and a screen between the heater and the pan. The screen was used to support the food during cooking and was adjustable to different distances from the heater, while the pan served to catch the drippings. The pan, screen, and heater could be lifted from the framework for cleaning. Initially, a 475 watt heater consisting of a coil of nichrome wire was used, but later it became necessary to shorten the wire in order to obtain enough heat to cook food in the helium atmosphere because of its high thermal conductivity. The degree of cooking was controlled by the amount of power supplied to the heater through a variable voltage transformer, and by the time of cooking. Under the conditions of the experiment, the food could be cooked on one side only, so, with the exception of suet, the volume of CO produced was doubled to represent cooking of both sides.

The quantities of CO formed during the cooking experiments are listed in table I. Although it is difficult to draw highly quantitative conclusions from these data because of the lack of reproducibility of the degree of cooking, they do indicate that certain relationships exist. Significant amounts of CO are given off by toasting bread and broiling meat. As one might expect, the quantity of CO produced is greater for a greater degree of cooking and is increased particularly by charring of the food. It appears that less CO is evolved, in general, by cooking in a helium - oxygen atmosphere with a low oxygen concentration than in air.

TABLE I  
CARBON MONOXIDE GENERATED FROM COOKING

FOOD	ATM.	P(PSIA)	DEGREE OF COOKING	CO GENERATED
Bread	Air	14.7	Dark brown	<0.2 cc/slice*
	Air	14.7	20% of surface charred	<0.2
	Air	14.7	75% of surface charred	1.8
	Air	14.7	75% of surface charred	4.3
	Air	14.7	75% charred completely through	34.0
	He-O <sub>2</sub> 2.2% O <sub>2</sub>	14.7	100% of surface charred through	19.0
	He-O <sub>2</sub> 1.4% O <sub>2</sub>	315	Dark brown	2.3
Steak	Air	14.7	25% of surface charred	30 cc/100g
	Air	14.7	100% of surface charred	70
	He-O <sub>2</sub> 2.1% O <sub>2</sub>	14.7	25% of surface charred	10
	He-O <sub>2</sub> 1.5% O <sub>2</sub>	315	Well done	10
	He-O <sub>2</sub> 1.45% O <sub>2</sub>	315	50% of surface charred	20
	He-O <sub>2</sub> 1.8% O <sub>2</sub>	315	Almost all surface charred	10
Suet	He-O <sub>2</sub> 1.5% O <sub>2</sub>	315	Melted, residue (<10%) charred	80

\*Average weight - 26g, average area 26 sq in (both sides)

One reason for the lack of reproducibility in cooking was variations in the thickness of the steaks and warping of the bread slices. Limitations of the apparatus required that the food be placed very near the heater and, as a consequence, the food was cooked unevenly with the thick portions and the high spots being heated more or charred to a greater depth than were the lower surfaces. Another uncontrollable variable was the change to a helium - oxygen atmosphere, which required a much greater power input to the heater because of the high thermal conductivity of the helium. These factors made it necessary to estimate the degree of cooking of the samples somewhat subjectively.

All the food cooked, with the exception of the bread samples which had 75% and 100% of their surfaces charred completely through, was considered edible. The surfaces of the other highly charred slices of bread would require scraping to be considered palatable. The steaks used were cube steaks and had almost no fat. They all were cooked at least well done, and none was charred to any great depth. The suet was sliced to approximately the size and shape of the steaks, and was heated on the drip pan, placed in the position where the screen had been for the other samples. During heating, practically all of the suet melted except for a small residue (<10%) resembling a mass of filaments or connective tissue that remained solid and was charred. Considerably more CO was given off by the suet than by the lean steaks. It is most likely that much more CO would have been given off by steaks containing an appreciable amount of fat than was obtained from the lean steaks used in the experiments.

### Activated Charcoal

Another possible source of CO in closed environmental systems is activated charcoal, commonly used for the removal of trace organic contaminants. It has been reported that chemisorbed oxygen forms a complex with carbon, which decomposes to yield both CO and CO<sub>2</sub> (Loebenstein and Dietz, 1955). These chemisorption complexes can be made to decompose by two methods, by an increase in temperature, and by removal of the decomposition products, such as by purging the carbon with gas or by evacuation. A study was carried out to determine if the amount of CO that might be contributed by an activated carbon bed is significant in actual practice.

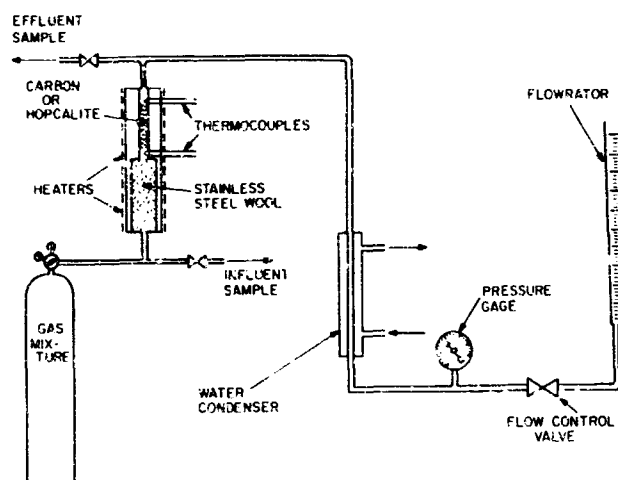


Figure 1. APPARATUS FOR STUDYING THE PRODUCTION OF CARBON MONOXIDE FROM CARBON

The apparatus used in this study is shown schematically in figure 1. Gas mixtures were passed through the reactor under various conditions, and the amount of CO evolved was measured. The reactor consisted of a 29-inch length of 1½-inch OD stainless steel tubing, mounted vertically, with the upper and lower sections enclosed in two independently controlled electrical heaters. The lower section, packed with stainless steel wool, served as a preheater. The upper section consisted of 7/16-inch ID tubing and contained a five inch deep bed of coconut charcoal of the type used aboard nuclear submarines. Thermocouples were used to measure both the inlet and outlet gas temperatures, and the temperature could be controlled to  $\pm 3$  F by adjusting the voltage applied to each heater. A water condenser was used to cool the gas before it reached the pressure gauge and analytical instruments. For experiments at high relative humidities, a water bubbler was placed between the gas cylinder and the reactor.

The carbon used weighed 7.1 g, and the same charge of carbon was used throughout this study. Parameters varied included type of gas mixture, temperature, pressure and relative humidity. The gas mixtures used were air and 1.5% oxygen in helium; pressures, 18.2 and 315 psia; relative humidities, 4% and 89%. The temperature of the bed was varied stepwise from room temperature to about 400 F.

A gas flow rate of 100 cc/min STP was used at both the low and high pressures, which is equivalent to a flow of about 3 CFM per square foot through a larger bed. The flow was relatively low as compared with those used in actual practice particularly at the high pressures where the linear gas velocity was one twentieth that at atmospheric pressure. However, it was expected that the lower flows should enhance the CO concentrations produced, and lead to more accurate analytical data. The CO was measured by means of gas chromatography, with a precision of about  $\pm 0.2$  ppm under the conditions used.

Figure 2 shows a typical profile of the CO concentrations found when the carbon charge was heated. During heating, and for a short time thereafter, CO was given off by the carbon. After the carbon had been maintained at an elevated temperature for a period of time, the CO level decreased, and after several hours, approached that concentration originally present in the gas.

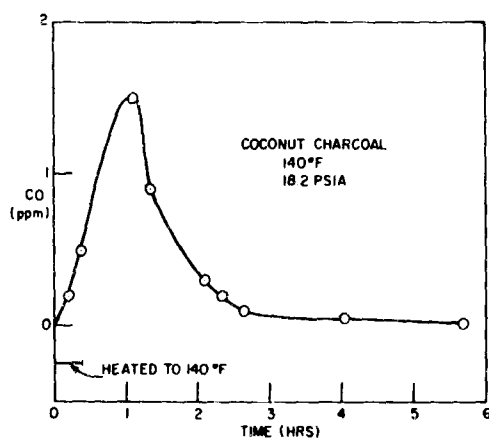


Figure 2. CARBON MONOXIDE EVOLVED FROM CARBON

Table II summarizes the data obtained under various conditions. The column, "Max CO Evolved", refers to the maximum of peaks similar to that shown in figure 2, while the "Min CO Evolved" values are those levels of CO given off by the carbon after several hours at temperature.

TABLE II  
CARBON MONOXIDE PRODUCED FROM ACTIVATED CARBON

<u>GAS MIXTURE</u>	<u>P(PSIA)</u>	<u>T °F</u>	<u>MAX. CO EVOLVED (ppm)</u>	<u>MIN. CO EVOLVED (ppm)</u>
98.5% He	18.2	75	<0.2	<0.2
1.5% O <sub>2</sub>	18.2	140	1.4	<0.2
4 % RH	18.2	198	5.4	<0.2
	18.2	270	55	2.0
	18.2	380	47	2.9
98.5% He	18.2	75	<0.2	<0.2
1.5% O <sub>2</sub>	18.2	140	1.5	<0.2
89% RH	18.2	212	1.4	<0.2
	18.2	320	6.7	0.3
	18.2	395	3.7	1.2
98.5% He	315	75	<0.2	<0.2
1.5% O <sub>2</sub>	315	143	<0.2	<0.2
89% RH	315	205	2.1	<0.2
Air	315	75	<0.2	<0.2
89% RH	315	150	<0.2	<0.2
	315	205	<0.2	<0.2

It can be seen from table II that very little CO is given off by carbon at temperatures at which a carbon bed normally would be used. Temperatures in excess of 200 F must be reached before CO production becomes appreciable. It might be noted that the temperatures used in this study are well below the ignition temperature of clean carbon in air, 650 - 750 F (Woods and Johnson, 1964).

No striking difference in CO formation between the experiments at 18.2 and 315 psia was noted. Although at the higher pressures, any CO formed would have been diluted more by the high pressure gas, the lower linear gas velocities used at these pressures should tend to compensate for the increased dilution of the CO. In the first two runs listed in table II, differing only in the relative humidity of the gas, a considerable difference in the amount of CO evolved was obtained. It is felt that the higher concentrations obtained in the first run were not due to the low relative humidity, but rather to the fact that this run was the first time that the carbon sample was heated,



after a long period of storage in an air atmosphere. Between ensuing runs, the time period was not long enough for the equilibrium between chemisorbed oxygen and the carbon complex to be established.

A much more detailed study would be required to determine the precise effect of these parameters on the rate of CO production from activated carbon. However, this study has shown that a carbon bed operated at ambient room temperatures would not be a significant source of CO in a hyperbaric atmosphere.

### Marine Sources

The sea itself is a potential source of CO in undersea habitats and personnel transfer capsules. It has been found that the amount of CO dissolved in the ocean is much greater than would be predicted from the equilibrium of atmospheric CO with the water (Swinerton, 1969). At a location near Trinidad, more than one hundred times the equilibrium concentration was found in surface water. Also, the amount of dissolved CO followed a diurnal cycle, and was greatest during daylight hours. At present, sufficient information is not available to determine the source of CO in the sea, but possible sources include the photochemical decomposition of organic matter near the surface, and the evolution of CO by marine life. Various algae, green plants, and some species of siphonophores are known to produce CO.

Atmospheric CO concentrations found by Swinerton averaged about 0.1 ppm over the open ocean. Thus a confined gas space, at one atmosphere pressure, in intimate contact with seawater containing one hundred times the equilibrium value, would contain 10 ppm of CO at equilibrium. Very few measurements of dissolved CO in seawater have been made as yet, and it is possible that other areas of the ocean could contain much more CO. Thus, the sea could contribute significant amounts of CO to the atmospheres of undersea capsules having an open interface with the water.

### REMOVAL OF CARBON MONOXIDE

Hopcalite was chosen for a study of the catalytic removal of CO from high pressure atmospheres because of its long history of successful performance in the atmosphere purification systems of nuclear submarines. In particular, it is noted for its resistance to poisoning, or loss of effectiveness in use. However, it was not known how well Hopcalite would perform at 20 atmospheres pressure in a predominantly helium atmosphere containing only a few percent of oxygen. A laboratory study was carried out to determine if the catalyst was effective under these conditions.

The apparatus used was basically the same as shown in figure 1. Instead of carbon, the reactor was filled with a bed of Hopcalite 7/16 inches in diameter and 5 inches deep, supported on a circular section of stainless steel screen. This part of the tube was heated with a 600 watt nichrome heater and the whole assembly was insulated with a glass blanket and asbestos tape. The space velocity used was the same as is used in operating submarines,  $21,000 \text{ hr}^{-1}$ . To maintain this space velocity at

300 psig, the laboratory bed required 3.2 CFM of gas as measured at atmospheric pressure and room temperature. If the bed had been at atmospheric pressure, the gas flow for this space velocity would have been only 0.15 CFM.

Helium containing 1.7% oxygen and 28 ppm of CO was passed through the catalytic burner at 300 psig and 600 F, and no CO could be detected in the effluent. Because of the high gas consumption at 300 psig, the high pressure run was terminated after 25 minutes, and further testing was done at atmospheric pressure. The same gas mixture was passed through the burner for a total of 37 hours, and no CO was detected in the effluent during this period. The study indicated that it was entirely feasible to remove the CO from a habitat such as SEALAB III by the use of a catalytic burner charged with Hopcalite catalyst.

A catalytic burner for use in SEALAB III was built by the Mine Defense Laboratory, based upon the laboratory study. Hyperbaric helium atmospheres cause problems in the design of an efficient burner. The high pressure gas requires about 13 times as much heat to raise its temperature to 600 F as does air at one atmosphere, and heat losses are greatly enhanced because of the high thermal conductivity of the helium. The SEALAB III burner, with an efficient heat exchanger and a well insulated housing, was capable of handling 3.1 CFM of gas at 20 atmospheres pressure and at 600 F with a power input of 2.4 KW. Tests in a pressure chamber proved the burner to be efficient for the removal of CO.

#### ANALYSIS FOR CARBON MONOXIDE

As man becomes subjected to environments at higher and higher pressures the analysis for CO becomes increasingly difficult. Since hyperbaric atmospheres contain approximately the same partial pressure of oxygen that is found in air at one atmosphere, the physiological effects of CO are directly related to its partial pressure. Thus the concentration of CO that must be measured becomes increasingly lower as the total pressure of the system becomes greater. For long term exposures of men to pressures of 20 atmospheres, the concentration of CO must be maintained below 1-2 ppm. Accurate methods of analysis are required to aid in the control of CO at these low levels.

The laboratory method used for analyzing for CO involves its chromatographic separation, catalytic conversion to methane, and measurement in a hydrogen flame detector (Porter and Volman, 1962). A Beckman GC-2A gas chromatograph equipped with a 6-ft x  $\frac{1}{4}$ -in molecular sieve column and a hydrogen flame detector was used for the CO analyses in this work. A two inch long tube ( $\frac{1}{4}$  - in OD) containing a nickel catalyst was added between the column and the detector, and the hydrogen used for the flame detector was diverted to a tee between the column and the catalyst. The catalyst was maintained at 300-350 C by means of an electrical heater.

When the system was first tried, difficulties were encountered with a wandering baseline and high noise levels associated with high background currents in the flame. Other workers had encountered similar troubles in trying to detect low levels of CO by this method. It was found that the difficulties encountered were caused by other contaminants in the samples, particularly carbon dioxide because of its relatively high concentration, and to a lesser extent, hydrocarbons. These substances eluted from the molecular sieve after many hours and caused baseline drift and the high background current.

A precut column was added to the system to prevent organic substances, and carbon dioxide which is reduced to organic compounds by the catalyst, from entering the molecular sieve column. A schematic diagram of the system used is shown in figure 3. Upon actuation of the 10-port sampling valve, the sample first enters the Porapak-Q precut column. Immediately after the CO is eluted from this column and enters the molecular sieve, the valve is restored to its original position, and carbon dioxide and other contaminants are backflushed from the precut column by a secondary helium stream. With this system, and the use of hydrogen diffused through palladium and air purified by passage over a hot palladium catalyst, a full scale response on a 1 mv recorder could be obtained for 1 ppm of CO, with a noise level of less than one percent, and almost no baseline drift.

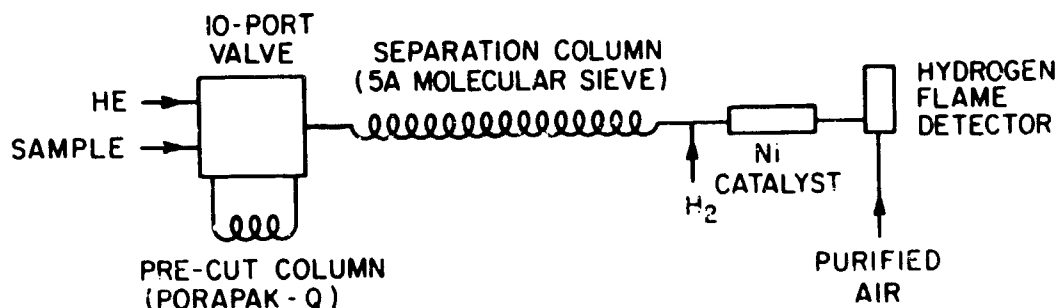


Figure 3. CHROMATOGRAPHIC SYSTEM FOR THE ANALYSIS FOR CARBON MONOXIDE

The precut column also eliminates certain branched hydrocarbons and some chlorofluorocarbons that can interfere with the CO peak or be mistaken for CO. These substances have molecular dimensions that are too large to allow them to enter the fine pores and cavities in the molecular sieve structure. Thus because of their size, they can be eluted from a molecular sieve column at about the same time as CO even though they are much less volatile.

The hydrogenation method for CO was quite cumbersome for field work because of the three gases required and the need for maintaining an active catalyst. Because previous work at this Laboratory with the Karmen helium dc breakdown detector (Karmen and Bowman, 1962) had indicated that this detector appeared to be inherently more sensitive for CO than the flame detector, and it required only a single gas, helium, for its operation, a chromatograph equipped with this detector was modified for use as a continuous CO monitor for SEALAB III. The column system in this unit was similar to that in figure 3, except that an additional valve was added in the middle of the molecular sieve column to divert oxygen and nitrogen from the detector. Without this valve, the CO peak appeared on a steep slope caused by the tail of the nitrogen peak. The system was designed to automatically analyze the SEALAB atmosphere, via a sampling line from the habitat, each half hour. Full scale response was obtained for less than one part per million with a negligible noise level. Further work is being carried out on this detector.

#### SUMMARY

A study has been carried out on some potential sources of carbon monoxide in hyperbaric atmospheres, its analysis, and its removal from these environments. Toasting bread and broiling meat has been shown to produce significant quantities of this gas in a closed atmosphere. Activated charcoal beds when operated near room temperature produce very little CO by reactions of the carbon with atmospheric oxygen. However, significant amounts of CO can be produced if the carbon is heated much in excess of 200 F. Dissolved CO in the ocean could be a source of this gas in the atmosphere of undersea capsules having an open interface with the sea. Hopcalite catalyst operated at 600 F is an effective means of removing CO from high pressure helium atmospheres containing low oxygen concentration. By the use of gas chromatography, sensitive methods have been developed for the analysis for CO at the one part per million level.

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## DISCUSSION

MR. WANDS: I just thought that it might be useful for some of these people who are involved in equipment design, in engineering planning purposes, to give them a figure for the amount of carbon monoxide released from the charcoal per gram of charcoal. The graph did not convert that way very readily.

DR. UMSTEAD (U.S. Naval Research Laboratory): We didn't calculate it up, because the only place we got significant amounts was at much higher temperatures than we were interested in anyway.

DR. LEON: The human produces about 0.4 cc of carbon monoxide per hour and, depending upon the size of the enclosure and the length of exposure, if you have enough people in the box, the CO will continually rise and if you are not removing it will cause a considerable buildup.

DR. UMSTEAD: Yes, I intended to mention that, but it is still much less than we found in Sea Lab. I think it amounts to about 10 to 20 ccs per man per day. There were ten men in the chamber, so it would only be about one tenth.

MR. MOBERG: We seem to get a leveling off of the CO in some of the spacecraft studies. I don't know if Dr. Harris would want to make a comment on this, but this seemed to be so from the measurement in the Apollo-7. Would you like to make any comment on this, Elliott?

DR. HARRIS: Actually, in these studies we haven't gone long enough to see whether we are continuing to buildup or not. In unmanned studies that we have run we find it will get to be 3 to 4 parts per million during the unmanned phase of the checkout of the spacecraft. That will be maximum. The men get in there, the CO starts up, and as I say, in those cases where we have had chamber runs at MSC, it seems to plateau out at around 28 to 30 parts per million at 5 PSI. This may have been due to spacecraft leak or it may have been due to other factors. We can't pin it all down, but it didn't go beyond that.

DR. UMSTEAD: We noted this in diving chambers, too. For the first 12 hours or so you get a very rapid rise in CO which I would think would be about equivalent to that at surface pressure. I attribute that to a lot of men being smokers, and when they got inside, once they get the carbon monoxide pretty well out of their blood it tends to level off for a while.

AMRL-TR-69-130

DR. HARRIS: May I make one other comment? Conkle and others at Brooks are doing some manned studies. You are involved in that too, Bud. Over a period of two or three weeks, with continuous analysis of the chamber, there was a steady buildup of carbon monoxide, and when you went back and calculated it, you found that it came out to an amount of 0.4 ccs per hour per man, a rate which fit very nicely with Dr. Leon's figures.

## ANOTHER INCIDENT OF DICHLOROACETYLENE CONTAMINATION

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Three or four months before the recent Biosatellite was orbited, there was a simulated flight test of the entire cabin system including a monkey. During that ground test the monkey became extremely ill. It was necessary to abort the test and the monkey later died. The monkey had been well instrumented and the medical staff monitored his physical condition in some detail. Two or three days after the beginning of the test the monkey's performance and physical well-being began to deteriorate. His condition continually worsened until on the eighth day the test was aborted and the monkey was removed from the cabin.

The monkey exhibited a number of symptoms during this period, but for the purpose of this discussion the following are the most interesting. Early in the test the monkey lost his appetite and eventually stopped eating altogether. His water consumption went down and, of course, his urine production consequently decreased. The monkey vomited once in the cabin and several times after he had been removed. He also had difficulty tracking with his left eye. After the monkey had been removed from the cabin it was observed that he was unable to close his mouth around the water dispensing device or to suck from the nozzle. Since some of the symptoms were suggestive of dichloroacetylene poisoning, a sample of air from the cabin was brought to the Naval Research Laboratory for analysis.

Several techniques are used at NRL for the analyses of trace contaminants in air. Conventional gas chromatography, of course, is one. We also use a chromatograph coupled to a special microcoulometric detector, which is sensitive only to chlorinated and brominated compounds. This system, which is extremely sensitive, was described here last year by Dr. Johnson. We also use mass spectrometry and mass spectrometry combined with gas chromatography. This particular sample was examined first with the equipment sensitive only to chlorinated hydrocarbons.

A number of innocuous chlorocarbons were detected at low concentration. But at the retention time characteristic of dichloroacetylene only a very weak signal was indicated. At the time this sample was brought to NRL the chromatograph was not rigged in its most sensitive mode. The signal recorded corresponded to a dichloroacetylene concentration of 0.1 ppm. Trichloroethylene, which is the precursor of dichloroacetylene, was also detected but at a concentration of 0.3 ppm.



The sample was then examined mass spectrometrically. Here again dichloroacetylene was detected at 0.1 ppm, still barely above its threshold level of detectability. Trichloroethylene was also detected, again at a concentration of 0.3 ppm. A dichloroacetylene determination of 0.1 ppm, especially when right at the threshold level of detection, is not much to hang your hat on and some of you may doubt its presence. But there is additional supporting evidence for the presence of dichloroacetylene which can be inferred from other circumstances.

First is the fact that trichloroethylene was also detected in this sample at a concentration sufficiently above its minimum detectable level to permit an unambiguous identification. At temperatures above approximately 70 C, trichloroethylene reacts with LiOH to form dichloroacetylene. LiOH was used in the cabin as a carbon dioxide absorber. Heat from the additional reactions of water vapor and carbon dioxide raises the temperature of the LiOH to the requisite level. Given otherwise appropriate conditions, therefore, the presence of dichloroacetylene is at least possible whenever trichloroethylene is present.

A second circumstance supporting the finding of 0.1 ppm of dichloroacetylene in the original air sample is the fact that both dichloroacetylene and trichloroethylene were found in the desorbate which was recovered from the charcoal used in the cabin environmental control system. No dichloroacetylene was found on a sample of the same charcoal which had not been exposed. These charcoal analyses were done by Mr. M. L. Moberg of the Aerojet General Corporation. Mr. Moberg also does all of the Apollo charcoal analyses. Since air flowing through the cabin environmental control system passes first through the activated charcoal and then through the LiOH, the dichloroacetylene which was adsorbed on the charcoal must have previously existed in the cabin atmosphere.

A third circumstance supporting the presence of a trace of dichloroacetylene was the reaction of the primate himself to the atmosphere in question. Unfortunately, the symptoms a monkey would exhibit as a result of dichloroacetylene toxicity are not known. But let's compare the symptoms displayed by this particular monkey to the symptoms that are exhibited by man to the same toxicant. A man's symptoms develop in the following order; anorexia, nausea, vomiting, headaches, and symptoms involving the facial muscles including painful gums and jaws. One to two days following severe exposures man additionally develops lesions around the mouth and lips. For less than fatal exposures, the time required for complete recovery in man seems to be proportional to the severity of the exposure. Complete recovery from severe exposures might require several months. Many of the outward symptoms displayed by man result from the effect the toxicant has on the trigeminal nerve. Man's loss of desire to eat and drink, for example, results from the desensitization of the oral nerve endings and the numbing of the tongue and inner mouth surfaces. The monkey exhibited some of these same symptoms. For example, a loss of appetite, nausea, and vomiting. The other symptoms are subjective. It's difficult to tell, for instance, if the animal has a headache. Sometimes the presence of a subjective symptom can be inferred, however. For example, when the monkey was removed from the cabin he was apparently unable to close his mouth around the drinking device and suck water from the nozzle, a fact which might have indicated

he had sore gums and jaws. In my opinion, the symptoms displayed by the monkey were indeed suggestive of dichloroacetylene poisoning.

How dichloroacetylene might have developed in this atmosphere is a puzzler in spite of the fact that all the necessary elements were present. Your first question, probably, is how did the trichloroethylene get through the charcoal to react with the LiOH? Only 10% of the cabin air circulated passed through the contaminant control system. The system contained both LiOH and charcoal, but the air stream passed first through the charcoal. If the trichloroethylene had originated from a source in the cabin it would have been retained on the charcoal and never would have reached the LiOH. It is unlikely that the trichloroethylene passed through the charcoal as a result of channeling. The charcoal was tested in the laboratory and found to be clean and moreover, to have ample capacity to retain much larger quantities of trichloroethylene than would have been encountered. However, the trichloroethylene might have off-gassed from contaminated surfaces in the LiOH unit itself. If so, there would have been an initial large production of dichloroacetylene when the unit was first activated. This would only have been eliminated gradually over a period of several days because of the 90% by-pass feature. Whereas the concentration might have been as high as several parts per million at first, it would have been reduced after several days to the 0.1 ppm actually found. This would have come about as the trichloroethylene was consumed and the dichloroacetylene was irreversibly eliminated by the monkey and by the charcoal.

After the first monkey was removed from the cabin a second monkey was inserted and the test was continued without incident for an additional 23 days. Air analyses during this second period indicated that the concentration of trichloroethylene decreased steadily from the first measured value of 0.3 ppm and never again reached a significant level. Dichloroacetylene was never again detected in any sample after the first.

Another point which may appear questionable is whether or not a concentration of 0.1 ppm of dichloroacetylene could have produced the symptoms displayed by the monkey. Captain Siegel of the Navy Toxicology Unit at Bethesda has been conducting toxicity studies with this compound for two or three years. His studies are still in progress, but results so far indicate that seven day exposures to dichloroacetylene at 7 ppm are 100% fatal to monkeys. Toxicologists usually measure toxicity only in terms of the number of fatalities. So any subjective and objective symptoms short of death those monkeys may have experienced have not been reported and may not even have been noticed. It would be interesting to investigate the symptomatological effects of a high initial concentration of dichloroacetylene which was steadily reduced to zero by the end of the exposure. Especially if the test monkeys were instrumented and closely observed, so that their physical conditions could be followed during the test. Perhaps dichloroacetylene at 0.1 ppm would have produced the distress the Project monkey experienced but, admittedly, it seems unlikely. Maybe 1 ppm or an even higher concentration would have been required. But as we have seen, the concentration might actually have been several parts per million during the early days of the exposure.

In my opinion, the actual and circumstantial evidence in this case seems to indicate that trichloroethylene was converted to dichloroacetylene in the test atmosphere during the first days of the experiment and the latter compound adversely affected the primate. This is the third time that serious consequences have followed from the presence of trace amounts of trichloroethylene in a closed atmosphere. These lessons are expensive. The next one might cost a life. So far 's closed atmospheres are concerned, "Let's stamp out trike!"

As a final thought, I would like to reemphasize that what we have been talking about here occurred during a ground test. The cabin atmosphere was free of harmful quantities of undesirable contaminants during the later orbital flight of the Biosatellite. So far as we now know, the cabin atmosphere during orbital flight in no way contributed to any of the space monkey's difficulties.

## DISCUSSION

MR. MOBERG: I think the comment might be made at the end that it wasn't attributable to dichloroacetylene but we are not sure that there weren't two or three other contaminants in the atmosphere that indirectly resulted in the demise or eventual demise of Bonnie, but we are not the pathologists or the medical people.

MR. SAUNDERS (U.S. Naval Research Laboratory): That wasn't the official reason given out.

MR. MOBERG: I just passed this as rumor.

MR. SAUNDERS: Atmospheric contaminants didn't play any part in it.

MR. MOBERG: We confirmed the data of the Naval Research Laboratory and the ratio that we might expect when you have lithium hydroxide present is about 5 to 1; for example, if you have about 5 milligrams per cubic meter of trichloroethylene and a good active lithium hydroxide removing the CO, you might expect the formation of somewhere around a tenth to two tenths of a milligram per cubic meter of dichloroacetylene. This seemed to follow the results of the analysis of the charcoal and the grab sample analysis.

FROM THE FLOOR: This is in the form of a question. It is possible that you might be able to approach this problem by loading the activated carbon, with the trichloroethylene and then use various contaminants or change the condition of the air going through this bed to find out whether there might be some material in the atmosphere that has passed through it subsequently to strip off the trichloroethylene which then would react with the lithium hydroxide?

MR. SAUNDERS: Yes, we performed an experiment just like that in the laboratory using the very charcoal that had been in the cabin. You can put a tremendous quantity of trichloroethylene onto the carbon and it will not elute off in any time short of 60 to 90 days. It is absolutely out of the question to assume that trichloroethylene had passed through the charcoal. The charcoal was clean to begin with and it was pretty clean after it came out, compared to what its normal full absorptive capacity would have been.

MR. MOBERG: Possibly I could add another comment here to more fully answer this. There might be materials that could displace trichloroethylene--benzene, toluene, etc. that might be more strongly absorbed than the trichloroethylene. There is nothing sacred about the binding or absorption on carbon. I think Ray's comments are well

taken because charcoal does have a large capacity for trichloroethylene but the capacity does change as other materials that are strongly absorbed pass through the charcoal and consequently elute.

MR. SAUNDERS: That isn't going to happen in any six or seven days, not with the quantity of charcoal they had in that bed.

MR. WANDS: These materials were not present?

MR. MOBERG: That is right, these weren't present, so it might be academic at this moment, but it still might be worthwhile to consider that there could be a displacement taking place.

MR. MITCHELL (North American Rockwell Corporation): I have a couple of questions. When they redid this experiment did they use a new charcoal canister with the second monkey?

MR. SAUNDERS: No, the same canister. They didn't remove the environmental control system at all.

MR. MITCHELL: So you had the same canister, the same charcoal, the same lithium hydroxide?

MR. SAUNDERS: Yes.

MR. MITCHELL: That eliminates one possibility. I thought maybe that this second canister was somewhat different from the first one. In that case, did you check the second canister, or other canisters? You hypothesized that the lithium hydroxide part of the canister may contain the trichloroethylene. Have you checked any other canisters to check and see if the lithium hydroxide was saturated?

MR. SAUNDERS: We were not able to check that particular canister because eventually at the end of the 30 day experiment it was removed, and later on, for some six or seven months during which time the lithium hydroxide charcoal had been removed, God knows what happened to the thing in the interim. But another canister lithium hydroxide unit was checked, and it was contaminated with tetrachloroethylene, which is a common cleaning solvent, you might say. Conceivably, the first one may have been cleaned in trichloroethylene.

QUESTION: Do the people that manufacture these have any comment on this? Do they use trichloroethylene?

MR. SAUNDERS: They deny everything.

MR. MITCHELL: I have one other question: This actually is probably to Bud Moberg. He mentioned that they have a certain ratio between trichloro and dichloro acetylene. Has this been borne out in the actual laboratory tests? Have you taken

trichloro, run it through a bed like this and produced 1/10 part per million?

MR. SAUNDERS: Yes, that has been done. Trichloro will convert.

MR. MITCHELL: In a straight lithium hydroxide bed?

MR. SAUNDERS: Right. Merle and his group have done it. So has Captain Seigel.

DR. LEON: I wasn't quite sure, did you say that in the official report of the bio-satellite that there were no contaminants in the atmosphere?

MR. SAUNDERS: Oh, there were plenty of contaminants, but they were not toxic.

MR. MOBERG: You didn't attribute that to the demise of Bonnie? The official report won't be available until January 10th.

DR. LEON: The official report says that Bonnie did not die of toxic contaminants, is that right?

MR. SAUNDERS: That was the version that I heard.

MR. MOBERG: It's really not official yet, it's tentative; that was carefully pointed out to me when I discussed this with the personnel.

DR. LEON: Is it also true that three of the controls died on the ground?

MR. SAUNDERS: No, I don't believe so, only one to my knowledge.

MAJOR CASEY: What was the autopsy finding on this monkey? Would you describe it as a sick monkey?

MR. SAUNDERS: Well, as to the cause of the death of the monkey during the ground tests, I don't know as it was given out. That would have to come from Ames. It was not due to dichloroacetylene poisoning.

MAJOR CASEY: How many implants, how many instruments did they have in it?

MR. SAUNDERS: About seventeen. If you think that fact had anything to do with its death they had many of their monkeys with that number of implants, and they went through the 30 day test without any difficulty.

MR. MOBERG: Certainly the implants would have some weakening effect on the animal, but I hardly think that you could get the symptoms of this dichloroacetylene toxicology.

FROM THE FLOOR: That's the point, you've just got a sick monkey. When a monkey gets sick he quits eating and drinking.

MR. MOBERG: Didn't you say something about closure of the mouth and so forth? It was more than just not feeling well.

MR. SAUNDERS: We are not hanging the dichloroacetylene on the symptoms displayed by the monkey. This was picked up by actual analysis, don't forget, by two different laboratories.

MR. MOBERG: I'm afraid for the official answer we will have to wait until next year.

MR. SAUNDERS: It shows you what an impact a very low concentration of dichloroacetylene can make in any experiment.

DR. MAC FARLAND: As a crusty old professor who edits a couple of toxicological journals, let me point out that no man has ever seen a symptom in a monkey. You may observe some signs.

MR. MOBERG: We will defend ourselves by saying we are chemists.

## ALIEN CONSTITUENTS IN EXPERIMENTAL ATMOSPHERES

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### INTRODUCTION

Despite increased emphasis on inhalation toxicology in recent years it has not seemed standard practice to monitor experimental atmospheres for unexpected or unintended constituents. However, composition of atmospheres for animal exposure studies, even in simple design systems, may be quite different from expected. While little has been reported in this area in toxicologic literature, it should be recognized that physical and/or chemical interactions may occur in gas, liquid, or solid phase among pollutants at any point in the exposure system (e. g., generation, distribution, exposure chamber), and between pollutants or products and the animals themselves in the chamber, resulting in undesirable effects on the primary study agent(s) and/or formation of new and alien gaseous or particulate constituents.

This report concerns examples of the actual occurrence of just such phenomena, some details of identification and quantitation of components of a resultant alien particulate, and mention of interest and speculation which arises concerning the influence of such alien material with respect to effects on the study subjects, study design and interpretation, and the power of extrapolation from the experimental exposure to a realistic exposure situation.

### MATERIALS AND METHODS

#### Nitrogen Dioxide Study

During a study involving exposure of dogs to an atmosphere containing relatively high concentrations of nitrogen dioxide ( $\text{NO}_2$ ) and ferric oxide particulate ( $\text{Fe}_2\text{O}_3$ ), the presence of unintentional particulate material was detected.



Exposure chambers, described by Hinners et al (1968), were of approximately  $2.83 \text{ m}^3$  (100 ft.<sup>3</sup>) capacity in the animal exposure portion and were constructed of glass and stainless steel with cubical dimensions of  $1.5 \times 1.5 \times 1.2 \text{ m}$  ( $5 \times 5 \times 4 \text{ ft.}$ ) and conical top and bottom. Chamber construction features provided a turbulent-mixing air flow pattern. Each was ventilated with a purified (CBR chemical and particulate-filtered), conditioned (70-75 F, 40-60% RH) air supply at the rate of  $1.4 \text{ m}^3/\text{min}$  (50 cfm), resulting in an atmosphere exchange rate of 30 per hour. Eight female pure-bred beagle dogs, each weighing approximately 11.3 kg (25 lbs) were exposed in each chamber.  $\text{NO}_2$ , generated by mixing dry filtered air with effluent from heated cylinders of 100%  $\text{NO}_2$ - $\text{N}_2\text{O}_4$ , and  $\text{Fe}_2\text{O}_3$  aerosol with a mass median diameter of  $0.54 \mu$  generated from commercial powdered  $\text{Fe}_2\text{O}_3$  in a device described by Crider et al (1968), were introduced at the chamber air inlet duct at a rate to produce concentrations of 20 to 30 ppm  $\text{NO}_2$  and approximately 0.8 to  $1.0 \text{ mg}/\text{m}^3$   $\text{Fe}_2\text{O}_3$  in the chamber at exposure level. The animal exposure pattern was established at about 22 hours per day, 7 days per week, for 6 to 8 months. Chamber atmospheres were assayed for  $\text{NO}_2$  by the method of Saltzman (1954) and monitored for  $\text{Fe}_2\text{O}_3$  aerosol by a Sinclair-Phoenix Forward Scattering Aerosol Analyzer, \*\* and flame emission aerosol monitor (Crider et al, 1968).

It was noticed during particulate monitoring in the initial phase of the study that the combined presence of dogs and  $\text{NO}_2$  contributed substantially to the aerosol loading in the chamber. Figure 1 shows the Sinclair-Phoenix response to the  $\text{NO}_2$  and dogs in a clean chamber. This extraneous aerosol response was more than twice that which represented the  $\text{Fe}_2\text{O}_3$  concentration to be used in the toxicity study.

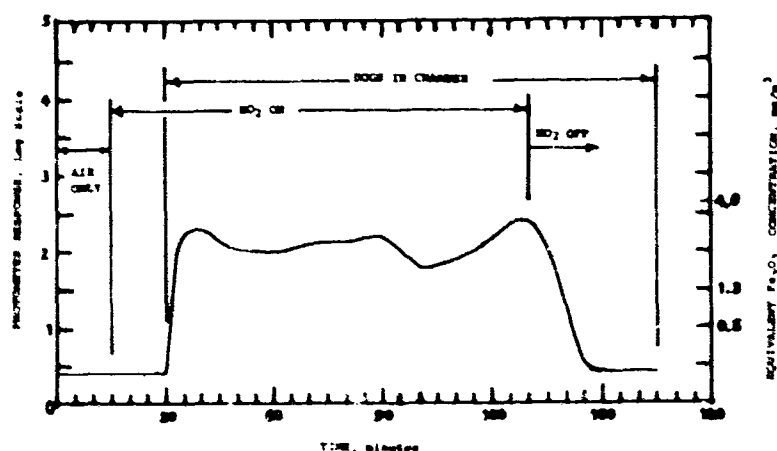


Figure 1. AEROSOL PHOTOMETER RESPONSE TO CHAMBER ATMOSPHERE ( $\text{Fe}_2\text{O}_3$  OFF)

\*\*Mention of commercial products does not necessarily constitute endorsement by the USPHS or DHEW.

In subsequent investigations of this phenomenon three factors were considered (presence of dogs, nitrogen dioxide, and  $\text{Fe}_2\text{O}_3$ ), combinations of which resulted in 8 chamber conditions: 1) no dogs or pollutants, filtered air supply only (designated 0); 2) dogs alone (D); 3)  $\text{NO}_2$  alone (N); 4)  $\text{Fe}_2\text{O}_3$  alone (F); 5) dogs +  $\text{NO}_2$  (DN); 6) dogs +  $\text{Fe}_2\text{O}_3$  (DF); 7)  $\text{NO}_2$  +  $\text{Fe}_2\text{O}_3$  (FN); 8) dogs +  $\text{NO}_2$  +  $\text{Fe}_2\text{O}_3$  (DFN). To minimize interruption of the toxicity study in progress, conditions were established from a fully operating chamber by removing the dogs and/or discontinuing generation of  $\text{NO}_2$  and/or  $\text{Fe}_2\text{O}_3$ . Time of removal was designated immediately as zero, beginning from which for each condition six consecutive 30 minute aerosol samples were collected from the chamber by constant-rate (7.4 lpm) pumping through acid washed glass fiber filters. Weight, pH, conductivity determinations and chemical analyses for ammonium, nitrate and nitrite were performed on all samples as described elsewhere (Knott and Malanchuk, 1969).

### Ozone Study

During a study to assess the inhalation toxicity of ozone ( $\text{O}_3$ ), and employing the same chambers (Hinnens et al, 1968), particulate counts from chambers were made under similar combinations of critical factors (with and without dogs and/or ozone) while controlling other potentially influential factors. The objective was to assess the particulate situation in relation to presence of dogs, ozone or both, compared to an empty chamber, with respect to enumeration and size distribution. In this work the particulate sampling, counting, and sizing was accomplished by use of an electronic particle counter. \*\* The study subjects were 8 female purebred beagle dogs, as in the previous ( $\text{NO}_2$ ) study. Ozone was generated from purified oxygen and introduced to the filtered, purified chamber air supply inlet so as to result in chamber concentrations in the range of 0.8 to 1.3 ppm. Chamber  $\text{O}_3$  concentrations were monitored by a Mast meter\* and by periodic wet chemical assay (neutral buffered KI), but no chemical characterization of aerosol was attempted.

### $\text{NO}_2/\text{O}_3$ Studies

In other work employing similar but smaller (approximately 0.34 m<sup>3</sup>) steel-glass animal chambers (Hinnens et al, 1968) concentrations of  $\text{O}_3$  and  $\text{NO}_2$ , when introduced singly and in combination, were determined for establishment of standard operating conditions to be used in animal exposure studies. While the  $\text{NO}_2$  -  $\text{O}_3$  assays did not involve animals in the chamber or aerosol collections/assays, interesting effects on the  $\text{O}_3$  and  $\text{NO}_2$  were demonstrated (Ulmer, 1967).

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\*\*Royco Instruments, Inc., Model 220

\*Mast Development Company

## RESULTS

Nitrogen Dioxide Study

In all analyses of samples of significant quantity, nitrate and ammonium ions were relatively abundant, while nitrite salts were absent. Compared to a reference curve (condition 0) of components, prepared by removing all generation and dogs at time zero and then sampling for 3 hours, curves of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  indicated the immediate beginning of chamber clearance so that the 30 to 180 and 150 to 180 minute sampling periods reasonably represented the equilibrium state resulting from removal of factors D, F, and/or N. Presence of dogs resulted in more rapid removal of the foreign aerosol material, indicated by measured  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , following discontinuation of  $\text{NO}_2$ ; i. e., these components decreased more abruptly when dogs were present, and at equilibrium (at end of sampling) were closer to zero in the FD and D conditions than in F and O, respectively.

When the chamber was receiving  $\text{NO}_2$ , the presence of dogs and  $\text{Fe}_2\text{O}_3$  gave approximately additive results, in terms of the ammonium nitrogen ( $\text{NH}_4 - \text{N}$ ) and nitrate nitrogen ( $\text{NO}_3 - \text{N}$ ) components of collected aerosol. This is suggested by the fact that, on a weight/volume basis,  $\text{NO}_3 - \text{NDN} \approx \text{NO}_3 - \text{NFN}$ ,  $\text{NH}_4 - \text{NDN} \approx \text{NH}_4 - \text{NFN}$ ,  $\text{NO}_3 -$

$\text{NDN} + \text{NO}_3 - \text{NFN}$ ,  $\text{NO}_3 - \text{NDFN}$ , and  $\text{NH}_4 - \text{NDN} + \text{NH}_4 - \text{NFN}$ ,  $\text{NH}_4 - \text{NDFN}$ ; also,

$(\text{NH}_4 + \text{NO}_3)_{\text{DN}} \approx (\text{NH}_4 + \text{NO}_3)_{\text{FN}}$  and  $(\text{NH}_4 + \text{NO}_3)_{\text{DN}} + (\text{NH}_4 + \text{NO}_3)_{\text{FN}} \approx (\text{NH}_4 + \text{NO}_3)_{\text{DFN}}$ .

Very little  $\text{NH}_4^+$  and  $\text{NO}_3^-$  was formed when  $\text{NO}_2$  was generated in the absence of both dogs and  $\text{Fe}_2\text{O}_3$ .

Based on sample averages over the 30-180 minute periods, the greatest absolute amounts of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were formed in the DFN, DN, and FN conditions, especially DFN; considerably less in the N and F conditions; and very little in the D, DF, and O conditions. Similarly, in terms of percent of total aerosol accounted for by assayed  $\text{NH}_4^+ + \text{NO}_3^-$ , the greatest proportion of aerosol accounted for by  $\text{NH}_4^+ + \text{NO}_3^-$  occurred in the DFN, DN, and FN conditions, especially DN.

Table I summarizes the average composition of chamber atmospheric particulate samples under the various conditions studied.

TABLE I  
AVERAGE COMPOSITION OF AEROSOL SAMPLES WITH  
VARIOUS CHAMBER CONDITIONS

Condition	Total Samples	NH <sub>4</sub> - N	NO <sub>3</sub> - N	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup> + NO <sub>3</sub> <sup>-</sup>
	μg/m <sup>3</sup>	μg/m <sup>3</sup>	μg/m <sup>3</sup>	% of Total	% of Total	% of Total
(1)	(2)	(3)	(3)	(4)	(4)	(4)
DFN	1962	$\frac{108.4}{98}$	$\frac{183.3}{150}$	5.7	34.4	40.1
DN	657	$\frac{51.2}{52}$	$\frac{106.0}{107}$	7.6	54.2	61.8
FN	1215	$\frac{39.4}{45}$	$\frac{86.4}{88}$	4.1	31.0	35.1
N	1814	$\frac{13.1}{8}$	$\frac{33.9}{29}$	0.1	5.9	6.0
DF	729	*	$\frac{17.2}{8}$	*	*	*
D	202	$\frac{3.3}{*}$	10.4	*	*	*
F	1030	$\frac{16.4}{6}$	$\frac{40.3}{35}$	0.6	13.7	14.3
0	*	*	*	*	*	*

(1) DFN = dogs, NO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> all present; DN = dogs and NO<sub>2</sub> only; FN = Fe<sub>2</sub>O<sub>3</sub> and NO<sub>2</sub> only; N = NO<sub>2</sub> only; DF = dogs and Fe<sub>2</sub>O<sub>3</sub> only; D = dogs only; F = Fe<sub>2</sub>O<sub>3</sub> only; 0 = no dogs, Fe<sub>2</sub>O<sub>3</sub> or NO<sub>2</sub> (empty chamber only).

(2) Based on 30-180 minute samples

(3) Upper figure based on 30-180 minute samples, lower on 150-180 minute samples.

(4) Based on 150-180 minute samples, % by weight of total sample.

\* Too small for analysis or significance.

Mass and molar relationships between ammonium and nitrate were examined. Graphs of  $\text{NH}_4^-$  - N and  $\text{NO}_3^-$  - N content of DFN condition samples through the 0 to 180 minute period demonstrate that the curve for  $\text{NH}_4^-$  - N closely follows the same pattern as the curve for its complementary  $\text{NO}_3^-$  - N (figure 2). In all sample sets  $\text{NO}_3^-$  - N content exceeded  $\text{NH}_4^-$  - N in average weight ratios approximating 2 (range of 1.6 - 2.3). On the basis of a 1:1 ratio of  $\text{NO}_3^-$  - N to  $\text{NH}_4^-$  - N, and of the relationships  $\text{NH}_4^-$  - N  $\times$  1.28 =  $\text{NH}_4$ ,  $\text{NO}_3^-$  - N  $\times$  4.43 =  $\text{NO}_3$ , and  $\text{NH}_4^-$   $\times$  4.43 =  $\text{NH}_4\text{NO}_3$ , weight percentages of  $\text{NH}_4^-$  +  $\text{NO}_3^-$  assayed accountable as  $\text{NH}_4\text{NO}_3$  ranged between 45 and 65 in those conditions yielding sufficient sample weights to be meaningful (F, FN, DN, DFN). As noted above, weight percentages of total particulate accountable as the sum of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , or ammonium nitrate, ranged up to 62% with the largest percentages being in conditions of  $\text{NO}_2$  present with dogs and/or  $\text{Fe}_2\text{O}_3$  (DN, FN, DFN, especially DN). It stands to reason that  $\text{NH}_4^+$  +  $\text{NO}_3^-$  would account for less total particulate when the intended particulate,  $\text{Fe}_2\text{O}_3$ , is present. Why so little total particulate is accounted for by nitrogen containing ions in the other conditions, especially N, is not clear.

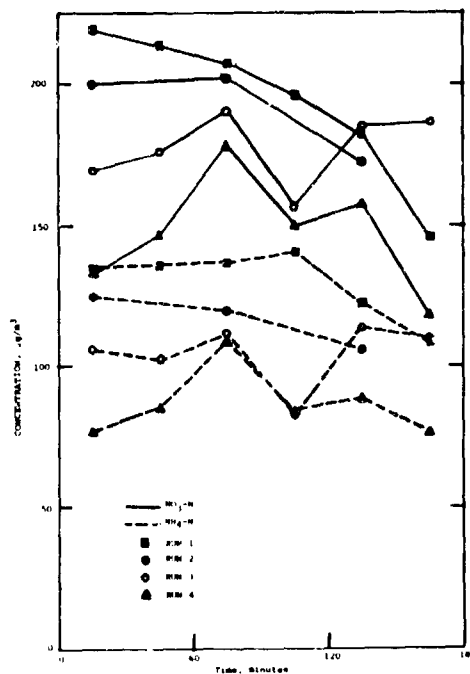


Figure 2. NITRATE AND AMMONIUM NITROGEN COMPONENTS IN CHAMBER WITH DOGS,  $\text{Fe}_2\text{O}_3$  AND  $\text{NO}_2$  (CONDITION DFN). Four sampling runs.

The observations clearly indicate the presence of particulate matter in excess of certain planned atmospheres, and suggest physical and/or chemical reactions(s) as a source. At least some of this is likely to be respirable. Under certain conditions a substantial portion of this alien material was nitrogen-bearing, and of this, ammonium nitrate,  $\text{NH}_4\text{NO}_3$ , probably assumed a major role. The nature of unaccounted-for nitrogen-containing and other total particulate was not determined but may be of sufficient interest for further study.

### Ozone Study

Total counts were obtained by the instrument for particulate size (diameter) categories:  $> 0.3\mu$ ,  $> 0.5\mu$ ,  $> 0.7\mu$ ,  $> 1\mu$ ,  $> 2\mu$ , and  $> 4\mu$ . For some of the collected data these were then corresponding derived categories:  $0.3-0.5\mu$ ,  $0.5-0.7\mu$ ,  $0.7-1\mu$ ,  $1-2\mu$ ,  $2-4\mu$ , and  $> 4\mu$ . Air was sampled from and data collected for: outdoors (designated OD; before air filtration and condition); in duct supplying air to chamber inlet (SD; after filtration and conditioning but before introduction of  $\text{O}_3$ ); and within the chamber under conditions of no dogs and no  $\text{O}_3$  (C),  $\text{O}_3$  but no dogs ( $\text{O}_3$ ), dogs but no  $\text{O}_3$  (D), and dogs with  $\text{O}_3$  ( $\text{DO}_3$ ). Outside and duct counts ensured that associated critical chamber counts could be compared for within-chamber effects of primary interest and were not being unduly influenced by rapid or severe fluctuations in outdoor-duct air aerosol content.

It was noted that outdoor air counts did fluctuate quite widely in time, and that this was reflected to some extent in the duct and chamber counts, specifically in the smaller size ranges ( $< 1\mu$ ). However, the proportionately small fluctuations in duct particulate count, their relatively slow temporal pattern, and frequent reference sampling along with chamber sampling, maximized the validity of chamber aerosol variations when dog- $\text{O}_3$  conditions were studied.

Chamber sampling indicated that without dogs in the chamber, the introduction of  $\text{O}_3$  in a quantity characteristically yielding approximately one ppm (0.8-1.3 ppm) in a dog-loaded chamber produced substantially greater concentrations (20-35%) than when dogs were present, and increased the total particulate counts by 18 to 73 percent. This suggests that dogs interact with and reduce the ozone introduced to their chamber atmosphere, which effect is noted commonly when  $\text{O}_3$  and other reactive agents and must be accounted for in characterizing chamber exposure conditions. It has also been observed that chamber materials (walls, inlet duct, etc.) react with  $\text{O}_3$ . Thus, it is not safe to assume that chamber animals are breathing the same concentration as sampled at the generator or chamber inlet duct. Also,  $\text{O}_3$  appears to react with chamber atmospheres even in the absence of dogs to produce alien particulate material in addition to that carried in the supply air, and in a respirable size range. Aerosol count increments of 18 to 73% were observed with  $\text{O}_3$  was introduced to chambers from which the dogs had been removed. With dogs in the chamber, introduction of the same  $\text{O}_3$  levels increased total particulate counts 11 to 81%. The total count pattern also appeared to follow that of  $\text{O}_3$  concentration when the latter was manipulated both with and without dogs. It was quite apparent that despite animal loading, introduction of gaseous  $\text{O}_3$  resulted in reactions yielding particulate matter alien to the planned atmosphere, in a manner similar to that observed in the  $\text{NO}_2$  study reported above.

That dogs alone contribute some to the chamber particulate loading was indicated by comparing ratios of chamber: duct counts and chamber counts with: without dogs, in the absence of  $O_3$ . Small to moderate increases in total particulate were observed to result from substantial surges in activity of the dogs in the chamber. This variable was accounted for, also, in relating particulate counts to other factors. In general, the dogs' presence appeared to contribute less significantly than did the introduction of  $O_3$ .

In addition to its effects in terms of total counts, i. e., all particles  $> 0.3\mu$ , introduction of  $O_3$  also was suspected of altering the size distribution of chamber particles. There appeared to be relative enhancement in the  $0.5-0.7\mu$  and possibly the  $0.5-1.0\mu$  range(s), and decreases in smaller and larger size categories, as a result of the presence of  $O_3$ . Comparisons of D and SD suspended particulate size distribution suggested that the presence of dogs increased the number of particles larger than  $0.5\mu$  diameter (especially  $> 1.0\mu$ ), and slightly decreased the number of particles in the  $0.3-0.5\mu$  category.

Frequency distributions of chamber and duct air particulates were heavily skewed to the  $0.3-0.5\mu$  extreme, with numerical median diameter (NMD) of approximately 0.43 and 0.4 respectively. These were in contrast to outside air particulates, which were distributed much more in the central size categories ( $0.5$  to  $2.0\mu$ ) and yielded an NMD of about  $0.7\mu$ . Quite obviously particle removal forces (filtration, sedimentation, impaction, etc.) in the air supply system were more efficient for the larger particles than for the smaller. More significant is the evidence that in addition to increasing total particulate loading  $O_3$  favored the enhancement of particulates in the deeply respirable size range.

#### $NO_2/O_3$ Studies

While no appreciable chamber loss was observed with  $O_3$  and  $NO_2$  introduced singly, when introduced in combination  $O_3$  was reduced by 63 and 69% at expected concentrations of 1.1 and 3.2 ppm, and corresponding  $NO_2$  losses were 13 and 31% at expected concentrations of 9.9 and 14.6 ppm, respectively (Ulmer, 1967). These coincident losses were characterized by  $NO_2/O_3$  molar ratios approximating 2.0, agreeing with the theoretical chemical interaction:  $2 NO_2 + O_3 \rightarrow N_2O_5 + O_2$ . Although no aerosol studies were made in conjunction with this work, it does demonstrate production of new, additional constituents alien to the original design. That alien particulate reaction products could also result, especially in the presence of animals, might be anticipated from the foregoing  $NO_2$  and  $O_3$  observations.

## DISCUSSION

The above studies present grounds for query and conjecture in regard to inhalation toxicology and its objective of extrapolation to other subjects and circumstances, and invite further, more detailed investigation to the extent that implications may be deemed significant. How universal is the phenomenon of atmospheric reaction and formation of new, alien particulate or gaseous products? What conditions control these reactions? To what extent might these affect interpretation of biological responses and the experiment as originally conceived? That toxicity may be influenced, especially potentiated, by addition of particulate (especially submicron) or gaseous agents, and some of the mechanisms by which this occurs, is already recognized (Amdur, 1957, 1959). Of some new concern, beyond this more obvious and direct consequence, is the question concerning what effect these alien constituents may have on the use and extrapolation of effects from an experiment involving these phenomena to a situation in which these may not occur, or occur in a different way and with different results. For example, could results of a laboratory chamber toxicity study of gaseous agents, involving appreciable alien constituents, effectively be extrapolated to humans in a community, home or space vehicle in which environmental conditions (concentration, ventilation, crowding, etc.) may alter or preclude the questionable alien constituents? These are subjects for further consideration and research efforts.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance of Messrs. Edward S. Thompson and Frederick J. Miller, DHER, CPEHS, NAPCA.

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## LIST OF SYMBOLS

Symbol or Abbreviation	Meaning
NO <sub>2</sub>	nitrogen dioxide
Fe <sub>2</sub> O <sub>3</sub>	ferric oxide (dust)
cu. ft.	cubic feet
cu. ft/min, dfm	cubic feet per minute
ppm	parts per million by volume
mg/m <sup>3</sup>	milligrams per cubic meter
NH <sub>4</sub> NO <sub>3</sub>	ammonium nitrate
m	meter
ft	lineal foot
m <sup>3</sup>	cubic meter
ft <sup>3</sup>	cubic foot
min	minute
μ	micron(s)
lpm	liters per minute
KI	potassium iodide (referring to assay method)
O <sub>3</sub>	ozone
O, D, N, F, DN, DF, FN, DFN	designations for chamber conditions, NO <sub>2</sub> study. See text.
NH <sub>4</sub> <sup>+</sup> , NH <sub>4</sub> <sup>+</sup>	ammonium (ion)
NO <sub>3</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup>	nitrate (ion)
NH <sub>4</sub> -N	ammonium nitrogen
NO <sub>3</sub> -N	nitrate nitrogen
%	percent
>	greater than
OD;SD;C, O <sub>3</sub> , D, DO <sub>3</sub>	designations for: outdoor; supply duct; chamber air and chamber conditions, O <sub>3</sub> study. See text.
μg/m <sup>3</sup>	micrograms per cubic meter
≈	approximately equal to

## DISCUSSION

MR. MOBERG: Let's go on then and let's look at the third paper, "Alien Constituents in Experimental Atmospheres". I think this should have provoked quite a bit of comment, particularly in the different reactions that could occur. One would assume that some materials are completely free from any noxious causatives, and yet if you have the particulates present such as Dr. Campbell pointed out, ferric oxide, you can form other materials. This is the kind of kinetics we were talking about, so that we can see what the products are that we are looking for.

DR. LONGLEY: I am curious as to what the air exchange rate in those chambers might have been.

DR. CAMPBELL: They run about 30 per hour, once every two minutes.

DR. LONGLEY: Also, where were you measuring the concentrations of the materials in the chamber?

DR. CAMPBELL: They were measured just above the level of the dog's head. We had them on the floor part of this chamber and then we had another shelf above them, about two to three feet above them, and that was taken just above that, so they couldn't fool with our sample probes.

DR. LONGLEY: Do you think increasing the flow of air would have eliminated some of what I think I'd call artifacts in your basic experiment?

DR. CAMPBELL: Perhaps, to some extent. I might speculate that, yes, if you increase the air exchange rate you might reduce some of these what I refer to as alien constituents but what might be called superfluous agents, but also conversely, I think if you had lower exchange rates, you would see more of this phenomenon. As to the artifact of this, I did mention that the presence of the dogs themselves contributes to these atmospheres. I fully admit that, but we do see these things forming, (and we first called them "doggie nitrates"), even in the absence of animals. There was another feature about this and it rather contributes to the idea that animal metabolic products are at the root of some of this. In some of our work we have had an opportunity to observe these reactions sometime after the animals had been in the chamber and find that we get less of this phenomenon. When the animals are freshly removed, there is still some residue of these metabolic products of one kind or another and I think that the longer they are out of the chamber, the longer that you have for really complete degassing--or whatever you would like to call it--an equilibration of the chambers toward a clean situation, I think you will see less and less of these effects. But our main

concern is with the effect with the animals in there, which is the environment that we are studying.

MR. VERNOT: Did you identify the material in the  $\text{NO}_x$  exposure as ammonium nitrate?

DR. CAMPBELL: We identified part of the material as ammonium nitrate. There was more nitrate material. As I mentioned, there were no nitrites found. We did find nitrates in excess of what could be accounted for by ammonium nitrate. If we take ammonium as the beginning point and multiply it by the factors to make ammonium nitrate out of it, we still haven't accounted for all the nitrate present, and when we add up all the ammonium plus nitrate, we have not yet accounted for all of the total particulate either, so we haven't answered the whole question. There is something else there, we don't know what it is. It will be very interesting and I think that more and more ideas will pop up as more people think about it.

DR. HODGE: I have two or three questions. I wonder if we could see the first slide on which you had data, the one that gave on the lefthand side of the slide total micrograms of particulate matter per cubic meter for the various experiments that you describe as D and F and N?

(Slide)

DR. CAMPBELL: I apologize for these, they are no better than some others.

DR. HODGE: The numbers that I would like to ask about, the top line D, F, N-- that's Dog and Nitrate and Iron all present, right?

DR. CAMPBELL: Yes, sir.

DR. HODGE: Total sample as I read it says 1962 micrograms per cubic meter.

DR. CAMPBELL: That is correct.

DR. HODGE: If we come down to the 5th line of N, which I take it means "Nitrate"--  $\text{NO}_x$  in the absence of Dog, it's 1814.

DR. CAMPBELL: Correct.

DR. HODGE: Does that mean that there is almost as much particulate matter when  $\text{NO}_x$  is there as if all three of them were there?

DR. CAMPBELL: Our preliminary conclusions lead us to believe that, yes. I should mention that I think these figures should be taken fairly relatively. They are actual measurements of total particulate mass, and they are averages of several runs, so there is some credibility to them, I believe, but you might think at first that there are some inconsistencies here: why that one for instance should be so high relative to the DFN condition or vice versa, why the DFN shouldn't be any greater than the "N"

condition. I can only suggest here that the presence of dogs has a way of removing some of these agents.

DR. HODGE: Right. You look at the DN, the DF, and the D, these are the three lowest numbers in your columns.

DR. CAMPBELL: Correct.

DR. HODGE: So the dogs are picking the stuff up, aren't they--picking it out of the air?

DR. CAMPBELL: Yes, they do.

DR. HODGE: Does this mean there are more particles, or are the particles heavier?

DR. CAMPBELL: Unfortunately, these studies here do not include any work on enumeration or sizing of particles. It's only in the ozone study that I did this, and that was done without the chemistry, so I apologize for this. We were rather in a hurry and this is rather a sideline observation that interested me. I don't know, I can't say. As I mentioned for the ozone study, the dogs themselves contributed to the particulate loading, especially in the greater than one micron diameter size range. So, that is about all I can say about that. I could only speculate similar things here.

DR. HODGE: And this increase in the number of larger particles might have been a growth of particles in the chamber?

DR. CAMPBELL: This is possible. The dynamics of this sort of thing we haven't investigated either. Certainly if you begin with gases being introduced into a chamber wherein there are gaseous agents and you end up with particulate agents, something is happening fairly quickly. The point I was making is that this sort of thing is occurring in our experimental situation, but it may be quite a different thing when we consider situations such as in a community or in a home situation or space capsule or some such thing, although I don't intend to get into the space business.

MR. MOBERG: Thank you very much.

DR. LONGLEY: I would like to ask one more quick one: What about the relative humidity, were you measuring it?

DR. CAMPBELL: We were measuring and controlling this in the region of about 40 to 60 at its practical extremes. It was held quite closely to 50% R. H. It wandered at times. We had trouble with our equipment and that sort of thing, but we do try to keep it right very close to 50.

DR. LONGLEY: In all these sets of exposures?

DR. CAMPBELL: Yes, I think we can say that it was 50 percent plus or minus ten percent. I understand your comment because this can be important in the production of particulate matter and in the nature of the particulate matter that is produced. This is well recognized and of course it just means more and more features of this thing that have to be defined.

## CONTAMINANT GENERATION BY ANIMALS IN THE THOMAS DOMES

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### INTRODUCTION

In toxicological exposures of large numbers of animals in the Thomas Domes, concern has been expressed for the possibility of toxic effects resulting from the build-up of contaminants from the animals themselves. This concern resulted in extensive discussion during past symposia of this series of the possible existence of unknown toxic materials in the dome atmosphere (Mautner, 1968; Weibel, 1968). This paper reviews some of the investigations that have been and are being made at the Aerospace Medical Research Laboratory to define qualitatively and quantitatively the existence of metabolic contaminants in the domes.

Most investigators concerned with the determination of contaminants in confined spaces have dealt with human subjects (Moberg, 1966) and gas-off products from construction materials (Hodgson and Pustinger, 1965). The list of products or contaminants found in inhabited confined spaces is quite formidable (Hine and Weir, 1965). The confinement of animals, however, has the additional aspect of biological waste as a contaminating factor. While there has been considerable work done on confined atmospheres, biological waste products have usually not been important, and atmospheric pollution from animal waste has received but little attention.

### EXPERIMENTAL

The information contained in this report was derived from dome studies involving dogs, monkeys, baboons, mice, and rats. The volume of the dome (figure 1) was approximately 800 cubic feet and the atmosphere used was 68% oxygen and 32% nitrogen maintained at 1/3 ambient pressure. The temperature was controlled at 70 F and the relative humidity at 50%.

To provide a clean atmosphere for the animals, the dome atmosphere was replaced at a flow rate of 120 cfm (of rarified air) or sufficient for a complete change-over every 7 minutes. The dome was thoroughly cleaned once a day and the floor flushed with water once or twice during each 8-hour shift period.

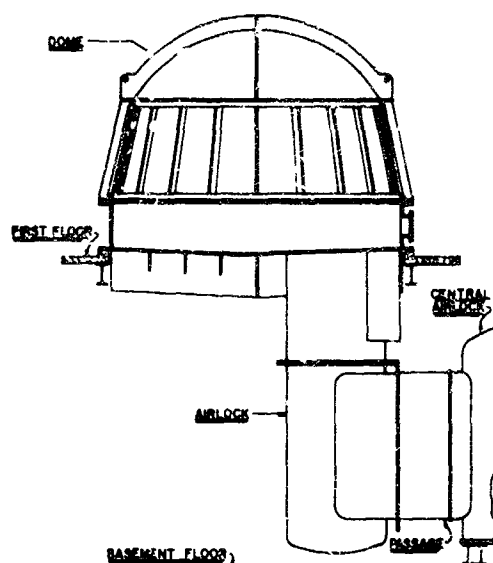


Figure 1. TYPICAL DOME ELEVATION

#### Analytical Procedures

To determine whether contaminants existed in a dome operated under these conditions, gas chromatography was the most sensitive technique available to us. Other techniques were tried but essentially without results. The presence of some alkaline materials was detected by passing the dome atmosphere through a standard acid solution and the presence of ammonia was verified by colorimetry. Otherwise, the method of choice was gas chromatography. The collection of samples required that a pump be used to withdraw atmosphere from the partial vacuum in the dome and pressurize it to ambient. When this was done, the sample became supersaturated with moisture so that we were confronted with liquid as well as gaseous samples.

A gas chromatograph equipped with a nickel hot wire thermal conductivity detector was used in preliminary studies. The nickel hot wire was less adversely affected by large concentrations of air than the usual tungsten detectors. We have found that with this instrument we can detect most materials at 100 ppm of any atmosphere. In these

dome studies, sufficient carbon monoxide was added to the atmosphere to provide a dome concentration of  $440 \text{ mg/m}^3$  which we were easily able to determine by direct injection into the chromatograph.

In addition to the chromatograph equipped with the thermal conductivity detector, other instruments equipped with flame ionization detectors were available. The flame ionization detector is much more sensitive to organic materials but is insensitive to any inorganic gas including carbon monoxide. Previous studies of confined atmospheres in which humans were involved indicated that methane was generated at a greater rate than most other gases (Auerback and Russel, 1966). Methane is also present at 5-15 ppm in the oxygen that is used in the dome atmosphere. The oxygen used in the domes has been monitored by another laboratory for methane, total hydrocarbons, nitrous oxide and halogenated compounds. Preliminary studies of the dome atmosphere involved direct injection into the gas chromatograph with the flame ionization detector. Only methane was detected by this technique.

#### Sample Concentration

We have used trapping techniques to concentrate the dome impurities, but the presence of large amounts of moisture has prevented this from being a straightforward procedure. Charcoal adsorption and direct cryogenic trapping with liquid nitrogen were the techniques used. Figure 2 shows a trapping system that permits the use of one or two pretraps to remove most of the water from our samples.

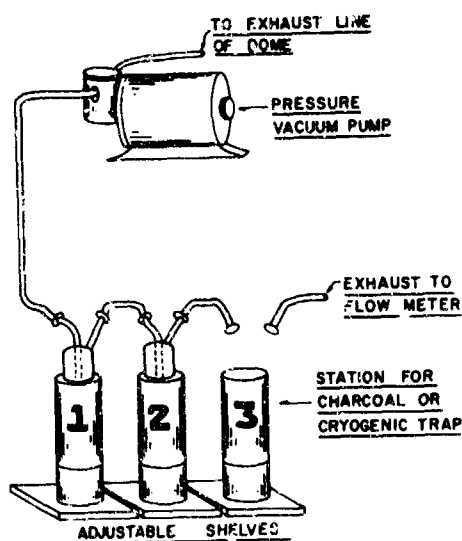


Figure 2. THREE STATION TRAPPING SYSTEM



## RESULTS AND DISCUSSION

Preliminary direct injections of dome atmosphere into the gas chromatographs served to indicate the level of contaminant concentrations to be expected. Since we could have detected most organic substances using the flame detector at a level of about 1 ppm or greater, we believe that there are no organic materials other than methane above 1 ppm in the dome atmosphere. In spite of the low level of materials present, there is nevertheless a disagreeable odor in the dome. W. E. Burnett (1969) points out that some materials of animal origin are capable of contributing odor at extremely low concentrations. Burnett used an effluent splitter with 20% of the effluent going into a flame ionization detector; the other 80% was sniffed by a laboratory worker who indicated odors as they came off the column. Figure 3 illustrates the gas chromatographic peaks and odors detected. Some materials produced odor but no peak.

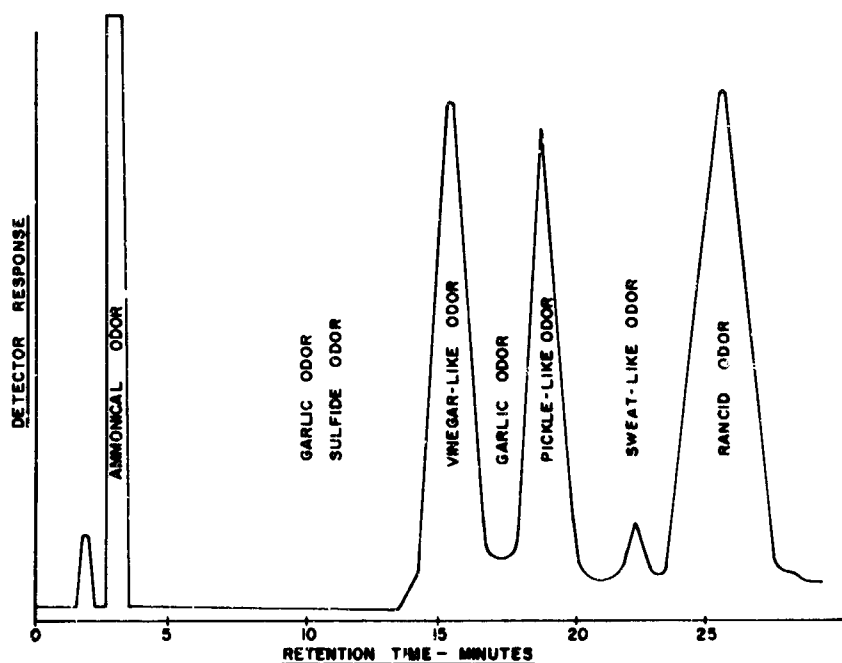


Figure 3. CHROMATOGRAM OF LIQUID MANURE VOLATILES WITH ORGANOLEPTIC EVALUATIONS OBTAINED FROM DIRECT INJECTION OF PREPARED SUPERNATANT. \*

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We recognized that we needed a sample concentrating device to obtain additional information. We used a charcoal trap that consisted of a 3/4" OD stainless steel tube 12" long. This tube was packed with 30 g of coconut charcoal and was equipped with fittings to allow installation in the trapping system or in a tube furnace for conditioning or desorbing. Trapping was usually done overnight in a system that utilized one or two pretraps. The desorbing was accomplished by vacuum distillation and the sample condensed in an ice water trap followed by a liquid nitrogen trap. Figure 4 and figure 5 are chromatograms prepared from the same sample trapping and are typical of those obtained by the charcoal technique. Several of the peaks have been attributed to low molecular weight hydrocarbons. The peaks attributed to acetone and methylene chloride were artifacts, however, having been introduced when some of the equipment was cleaned with these solvents. With our technique, the charcoal trap preferentially adsorbed low molecular weight nonpolar materials and, therefore, could not be depended upon alone for quantitative estimation of all the contaminants.

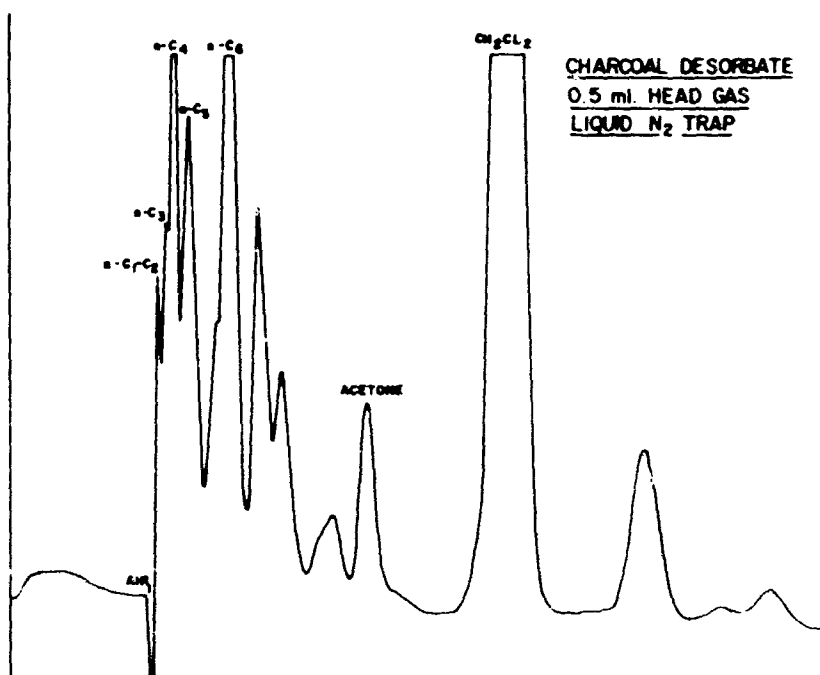


Figure 4. TYPICAL CHROMATOGRAM OBTAINED FROM HEAD GAS FROM CHARCOAL DESORPTION INTO LIQUID NITROGEN TRAP.

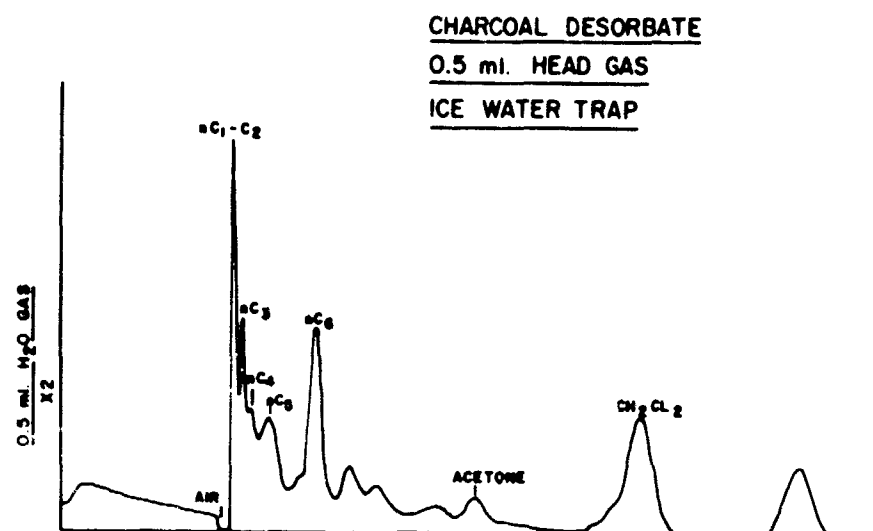


Figure 5. TYPICAL CHROMATOGRAM OBTAINED FROM HEAD GAS FROM CHARCOAL DESORPTION INTO ICE WATER TRAP.

One or two pretraps were always used to reduce water whenever samples were collected but the presence of water was always a problem. In addition to water, the liquid nitrogen trap also collected oxygen and carbon dioxide. On warming the trap, prior to obtaining a gas chromatograph sample, the oxygen, carbon dioxide, and most of the methane were lost. The remaining water and head gas from the samples were then examined and gas chromatograms similar to figures 6 and 7 obtained. Figure 7 shows a temperature programmed sample that produced 30 peaks. Not all the peaks produced were from materials generated in dome, however. Various materials were introduced from the outside air that was mixed with oxygen to produce the 68%  $O_2$  - 32%  $N_2$  dome atmosphere. On figure 7 we found that peaks 11, 21, 25, and 29 matched those found in outdoor air with respect to retention times and peak heights. Peak number 9 and a small portion of peak 23 were introduced by gas-off from the chromatograph septum. All peak identifications were made by matching retention times at several temperatures with those of known compounds. From day to day peak intensities varied but the total concentration remained very low. From our best estimates of peak areas, the total concentration of organic contaminants (aside from methane) is less than two parts per million of the dome atmosphere.

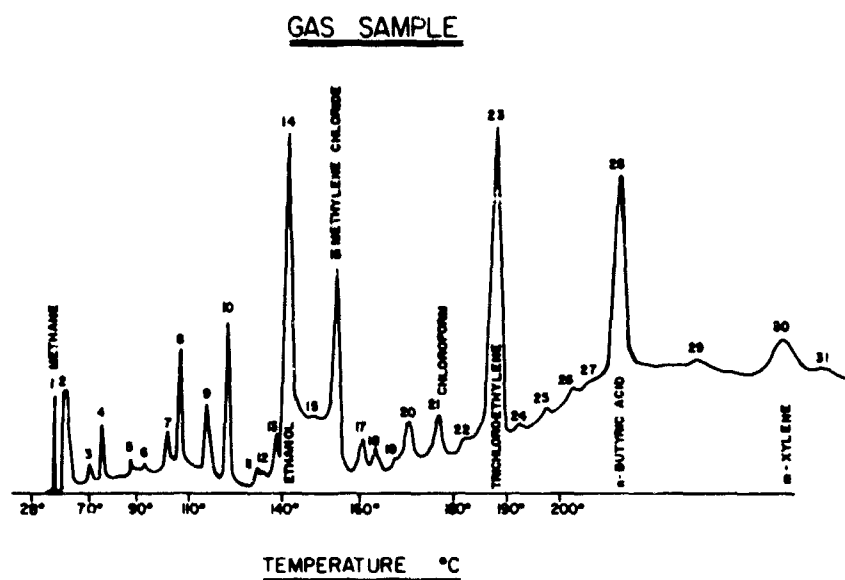


Figure 6. GAS CHROMATOGRAM OBTAINED FROM LIQUID RESIDUE IN LIQUID NITROGEN CRYOGENIC TRAP.

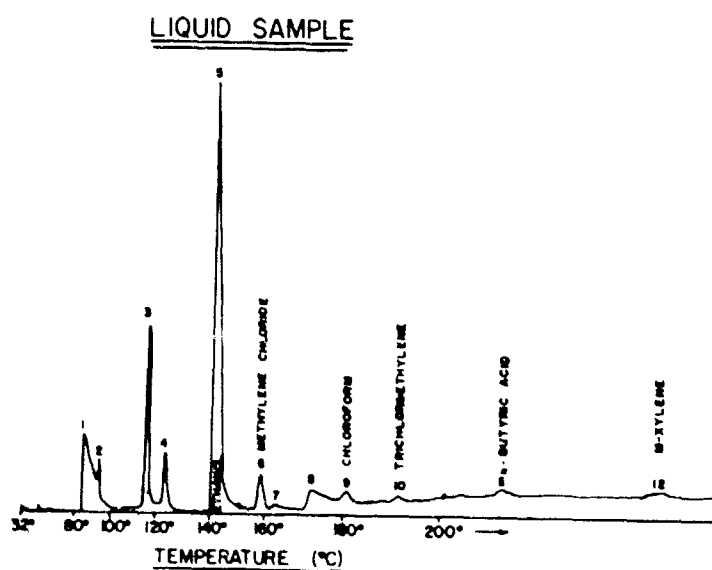


Figure 7. GAS CHROMATOGRAM OBTAINED FROM HEAD GAS IN LIQUID NITROGEN CRYOGENIC TRAP.

CONCLUSION

Table I is a tabulation of the threshold limit values (TLV) of some of the materials that we and other investigators in this field have found. Retention identification indicates that the dome contaminants are not highly toxic materials and are present in such extremely low concentrations that they should have no physiological effects on the experimental animals housed in these chambers.

TABLE I  
THRESHOLD LIMIT VALUES FOR SOME COMPOUNDS OF INTEREST

	<u>TLV</u>	<u>Found</u>
Methane	asphyxiant	17-19 ppm
Ammonia	50 ppm	0.50 ppm <sup>1</sup>
Chloroform	50 ppm	.035 ppm
Methylene Chloride	500 ppm	.040 ppm
Trichloroethylene	100 ppm	.010 ppm <sup>2</sup>
n-Butyric Acid	10 ppm	.015 ppm
Ethyl Alcohol	1000 ppm	0.25 ppm
m-Xylene	100 ppm	.010 ppm <sup>2</sup>
Ethyl Mercaptan	10 ppm	} not detected
Methyl Amine	10 ppm	
2-Butanone	200 ppm	
n-Propylacetate	200 ppm	
Ethyl Ether	400 ppm	
Acetaldehyde	200 ppm	

By utilizing the total peak area and the relative sensitivity of methane, the total organic vapor content of the dome atmosphere is estimated to be less than two parts per million.

<sup>1</sup>Estimated from alkalinity measurements.

<sup>2</sup>Peak area relative to methane.

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## DISCUSSION

MR. MOBERG: The other paper was "Contaminant Generation by Animals in the Thomas Domes" and maybe there are some questions that are appropriate at this time. I have some questions myself on this particular one. I'm not sure that we can call a cabin clean if there are no materials above the one part per million range because we are interested in the total picture of contaminants as far down as we can accurately measure and there seems to be significance in materials below parts per million levels, even in the control chambers. Now Doug might disagree with me on this, but I think it is worthwhile that we look at levels below a part per million and show that the cabin can be contaminated with materials which later might affect the experiment indirectly. Doug, would you like to comment on this?

DR. MAC EWEN: Yes. I'm not so sure that I quite understand what you are saying, but I think you are saying that we should be concerned with any of these materials regardless of how little is there. It is true that there are quite a variety of materials there, most of them originating from the animal and the concentrations of many of these were highly insignificant from a toxicological point of view. There were a few chlorinated hydrocarbons present which may come from the hypox paint that the dome is painted with on the inside. The dome is painted to prevent corrosion of the metal surfaces; again, the concentrations of these were extremely low and below, far below, recognized significant toxicological levels.

MR. MOBERG: All right. I was a little concerned with the one slide using the human detection system for identifying materials. I would be very interested in knowing what some of these components are. I think we have made some identities on such odorants, but before I would comment, I would really like to get some substantiation from other workers in the field. I don't mind sticking my neck out, but sometimes it is out so far that I can never withdraw it.

MR. VERNOT: I think I can say something about the paper from which this data was taken. In order to get gas chromatographic peaks for the materials that they were interested in, this was done on chicken droppings--is that right, John?

FROM THE FLOOR: That is right.

MR. VERNOT: And they couldn't get gas chromatographic peaks for materials they knew had to be in there because they smelled them and they were looking for things like indole, skatole and such which are normally associated with these things. So what they did was to make a stew out of the droppings themselves and looked for

them, not in the atmosphere, but in the solid and liquid mash that was actually lying on the ground. Only then could they get these gas chromatographic peaks for the materials which were so odoriferous that they were really overwhelming in the chicken yard or wherever they were keeping these animals. We didn't do that, of course, we had to work with the physical state of interest to us, the atmosphere. We weren't interested in the feces or the liquid state or this sort of thing because the animal really doesn't come in contact with these things. So there again, in order to get some of these odoriferous materials gas chromatographically present techniques are not advanced to the point where we can find them yet.

The other thing you mentioned was, as far as the organoleptic effects of some of these odoriferous materials, this is not a new technique. I know because I used to work for a food corporation and you get gas chromatographs of coffee aroma or fruit aroma, this sort of thing, and the first thing you think about, you get some expert nose as a detector, and it has happened time and time again that the nose can detect materials that the most sensitive gas chromatographic detector misses entirely--it just doesn't see it.

MR. MOBERG: Before I allow any more comments or questions back there, I'd like to challenge this in part. I'm not completely ready or able to say that we can identify all of these odors. There is a general feeling that detection by smell usually goes down to about .05-.08 PPM and then you really fall off even for the most odorous materials, even for these ketones. I'm not sure about that. I know you are ready to make a comment, but this has been reported in the past. Now I would offer this, that with detection techniques and back up of the GC with the mass spectrometer we are able to go at least a hundred times more sensitive than this and get some reasonably good qualitative data. It is possible that the odors are really a lock and key condition or a combination of three or four components which produce a recognized odor, garlic odor, or something of this sort, but still it is very important that the analytical chemist start unraveling these things so that it can aid the whole area of work.

MR. VERNOT: Well, I certainly agree with your thesis that the instrumental approach is the one to be taken, and of course we know that the detectors that have been developed recently are becoming more and more sensitive; however, it's not true that they have approached the sensitivity of organoleptic sensors to some of the extremely odoriferous substances. Not only can the nose detect parts per billion but it can detect parts per trillion.

MR. MOBERG: What is your technical backup for that?

MR. VERNOT: Well, I can't quote references at the moment, but as I say, having worked in the food industry some years ago, what people do is to make known dilutions, and it is not hard to do this. You can achieve a certain maximum concentration. You know that there is an upper limit to what you've got--people still smell it.

DR. LONDON (Aerospace Medical Research Laboratory): With respect to hydrazines, a paper was given at the recent AIA Meetings in Denver, and in a double blind



kind of experiment people were asked to stick their head in a plastic bag and record any observation they cared to, or else enter a room into which a given concentration of either UDMH or  $\text{NO}_2$  had been infused, and after--I guess there were about 10 or 15 people on the panel--and the numbers that were given as detectible routinely for UDMH was 3/10th of a part per million and a half of a part per million for  $\text{NO}_2$ . As I indicated, we don't have an instrument that can do that, anywhere near that reliability.

MR. VERNOT: That is true and it is also true that UDMH and  $\text{NO}_2$  are really not bad smellers when compared to some others.

DR. CAMPBELL: We have also used our smellers in the animal chambers and without hardly any more than mentioning what psychological factors might be involved in these things in chronic studies, we do smell some very strong odors in these animal chambers, when we just open them up in spite of the ventilation rates, on the order of what I have mentioned. Our chronic automobile exhaust studies in particular are this way, and even in the larger chambers that I described which are running at about twice the ventilation rate that the automobile exhaust exposures are running, we smell odors and I don't know what they are. They are a conglomeration of things, but even in control chambers we smell odors, or I think I smell odors that are quite different from what I smelled in the hospital lab. That's pretty well known to anybody. These odors do seem to be a bit different, and I'm not sure why. Of course these chambers are steel and we do get an accumulation of this material on the chamber, and there may be some metallic effect on these things, producing odd balls, but I think this must all be considered in a chamber situation. I might also add as a matter of new information on materials in our auto exhaust atmosphere; we initially wanted to be sure that sulphur dioxides, including sulphuric acid, were present in these atmospheres, combined with various types of exhaust atmospheres, and so we proceeded to add these, then we monitored the atmosphere and found that we did not need to add sulphur dioxide and sulphuric acid to these atmospheres. They were there in abundance in the order of magnitude that we wanted, so we had to drop that notion, and I think they are working on the whys and wherefores of this.

DR. THOMAS: This is really related to this alien material or whatever you want to call it from the toxicological standpoint. We are in the same boat as everybody is in inhalation toxicology. As our instrumental sensitivity increases, our previous limits of perfection become obsolete. Now there is an easy way out. If you run your experiments, run the proper controls, duplicate the same environment, then by subtraction of differences, you'll get the toxic effect. But I'm not so sure that what we see there is due to the chemical at all. It might be due to a biological agent. It might be due just to confinement. Any change of environment which is different from the usual environment--That's why we started preconditioning the animals in these chambers. I hate to use the word stress--but it is a different environment and the animal will adjust to that, and in that process of adjusting you will see all kinds of findings if you look closely enough. I just have a report on my desk from Major Coyne who is doing his PhD thesis on immunological responses due to confinement in space cabin atmospheres. He finds a high decrease in spleen weight, depressed immunological titer after challenge in 5 PSI oxygen. He went to 7.5 PSI mixed gas, found the same. So

I said, "Well put some animals in the dome at room air as controls." Well, there too, with no "contaminants", no oxygen but ambient air in the dome--is the depressed hematological response. Well, the only out is that you treat your control groups exactly as you treat the exposed ones. That's about all we can do.

MR. MOBERG: I have three slides that I would like to show you and some of the data was collected almost a year ago from the Apollo 7 and the data was reduced a little after this time, but it might be new to you, and we will very quickly look at these and I'll make a comment or two on them. May I have the first slide, please.

(Slide) What I wanted to show you was, this is a partial list of the materials that were identified and quantitated on the Apollo-7 preflight studies at Cape Kennedy and it is really just put up here to show you the large mass of data that was accumulated. Now I will go to the next slide.

(Slide) This is data that was taken from the charcoal from the Apollo-7 flight. It was a double blind experiment. The laboratory did not know the order of the canisters. They did not know the concentrations or gas flows through the charcoal. It does illustrate that trace analysis can be reproducible, and it shows some definite trends; and by the way, these numbers are absolute in relationship to each other. They are percentage relationships. For example, in the acetylene, the first little bump might be only five percent of the major mass peak, so that it does show reproducibility. This shows that there is a trend for propanol that was formed or liberated during the course of the experiment. The methylchloride was present probably as a degassing material all the way through the flight. We don't know where the acetylene came from but it certainly was present in the initial stages; maybe a product released from an electronic device and then the atmosphere eventually cleaned through the charcoal system later, and the same for the hydrocarbon or octane with the exception of this one peak. It may be real. We do not know that because it might have been released from an electronic component. May I have the next slide?

(Slide) That's just another example of random selection of different materials and then this is one of the alcohols, and you can see some trends again of the lower alcohols forming in the earlier part of the flight, or at least evident in the charcoals, and then the higher alcohols, and even the isopropanol quite high toward the end of the flight. Thank you for those slides.

In this analysis we used an electron capture detector. We used flame ionization detection, and at the present time we are using thermal conductivity analyses along with those other two, with the backup of mass spectrometry. We feed the effluent of the chromatographic system into the mass spectrometer, and we are able to see, on a relative basis for a material like taurine from a grab sample of about 250 cc, ten parts per billion extrapolated into the atmosphere sample, and the mass spectrometer can back me up to about four times this number, so that we do have a good excellent quantitative tool behind us in gas chromatography. One important thing we have learned is that when we get a peak, even though it has the same retention time of a signal that we collected the day before, the large number of organic compounds can potentially be present. There can be at least two, three, or four components in that single peak at

the same retention time. I used to be very strong on retention measurement. I still hold on to those for my quantitative data, but I must rely on the other detection techniques to give me more assurance of what this material is; and in many cases, I have found that the material has not been what I suspected it to be, and sometimes this is the way we find materials like monochloroacetylene when we think it is one of the freons, and we certainly wouldn't want to confuse the profession this way. But it easily can happen.

## CONTAMINATION IN A SEMICLOSED ENVIRONMENT - THE MODERN HOME

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### INTRODUCTION

Interest in the more subtle influences of environmental contamination is just beginning to be felt important enough to merit serious scientific investigation. This is largely due to the efforts of toxicological "splinter groups" concerned with atmospheres in unusual environments and with the effects (such as mutagenic changes) of long-term exposure to very low concentrations of contaminants. The importance of these subtle effects in the home environment where we spend the majority of our lives has been only casually treated in largely philosophical dissertations.

Rene Dubos follows this inclination when he lists some of the possible problems in "environmental biomedicine" (Ewald, 1967):

1. The lasting effects of early influences; i. e., of the effects exerted on the organism during the formative stages of its development.
2. The delayed and indirect effects of environmental pollutants.
3. The distant consequences of exposure to subcritical levels of potentially injurious substances and stimuli.
4. The effects of housing conditions on the development of sense organs and of various physiological processes.

For some time, it has been one of my desires to examine the possibility of significant environmental improvement in the home atmosphere. I was afforded this opportunity upon the initiation of a long-term study sponsored by the American Gas Association and The East Ohio Gas Company (to be described later).

### THE TREND TOWARD THE CLOSED ENVIRONMENT IN THE MODERN HOME

Advancing technologies in architecture and building construction and the new materials available make it possible, and for many economic reasons highly desirable, to virtually close the system with respect to the gaseous environment in the modern home. From the few pertinent studies on this subject it is clear that we

have decreased the amount of fresh air ventilation in homes and offices - particularly over the last 10 years. Considerable savings in the cost of heating and cooling the modern home can be achieved by these methods. Furthermore, those familiar with the air pollution problem in the Los Angeles basin know that the only respite from the uncomfortable outside world is a well-sealed, air-conditioned enclosure. Sealing oneself away from the polluted atmosphere is becoming more popular as it becomes apparent that effective solutions to air pollution problems can be anticipated only at some indefinite future time.

The space cabin and submarine are virtually closed systems; we feel that it is possible to handle any contamination of these environments with proper filtering and scrubbing techniques. Very seldom, however, are any suggestions made to similarly purify the atmosphere in the semiclosed home environment. Moreover, if we do provide fresh air ventilation in polluted areas, this too should be cleaned up.

Ventilation or the infiltration rate (expressed in air changes per hour) is a highly important but poorly calculated or measured parameter in the design considerations of architects and heating and air-conditioning engineers. Modern technical practices dictate a minimization of infiltration, especially when more costly energy sources are used (Edison Electric Institute, 1965; Ambrose, 1966; ASHRAE, 1967). For example, each 1000 CF/min reduction of outdoor air represents a savings of approximately 3 tons of refrigeration equipment and 80 million Btu/hr in heating equipment, and a corresponding annual operating cost savings of \$400 - \$500 in electrical energy consumption (Ambrose, 1966). Figure 1 shows the recommended infiltration rates to achieve various degrees of "pure" air.

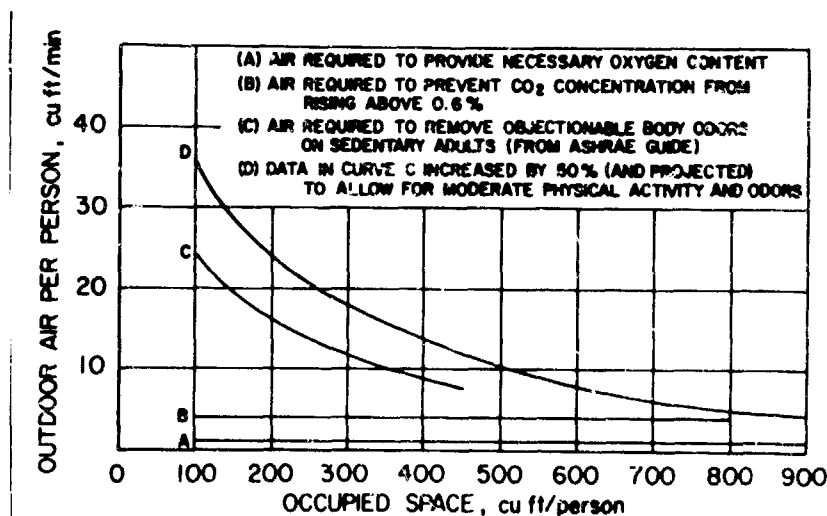


Figure 1. VENTILATION REQUIREMENTS RELATED TO CUBIC FEET OF OCCUPIED SPACE PER PERSON

Ideally, Curves A and B in figure 1 are the goals to achieve, but it was recognized that odors would need to be removed. Objectionable body odors are, in fact, the criterion for minimum ventilation requirements. Table I outlines the most commonly quoted minimum requirements found in the ASHRAE Handbook of Fundamentals (ASHRAE, 1967) and somehow formulated by Yaglou in 1936. It is most important to note here that all of these regulations for the total house are directed at reducing odors emanating from humans - not from processes or materials.

TABLE I  
MINIMUM OUTDOOR AIR REQUIREMENTS TO REMOVE  
OBJECTIONABLE BODY ODORS

Type of Occupants	Air Space per Person, cu ft	Outdoor Air Supply, CF/min per Person
Heating Season With or Without Recirculation:		
Sedentary Adults of Avg. Socioeconomic Status	100	25
	200	16
	300	12
	500	7
Laborers	200	23
Grade School Children of Avg. Socioeconomic Status	100	29
	200	21
	300	17
	500	11
Grade School Children of Lower Socioeconomic Status	200	38
Children Attending Private Grade Schools	100	22
Summer Season. Air Cooled and Dehumidified. Air Circulation 30 cu ft/min per Person:		Total
Sedentary Adults	200	<4

The range in fresh-air requirements runs from 5 to 25 CF/min per person. In most homes, this would amount to less than 0.5 air changes per hour. The readily available data demonstrate that this infiltration rate is often not achieved in even many of the older homes; in newer homes having an ordinary amount of insulation and using ordinary construction materials and methods, this rate is approached only in severely cold weather and high wind velocity conditions.

Very little is known about infiltration rates in homes. This parameter usually turns out to be included in the "error factor" in computing heating and air-conditioning calculations. Calculation methods are crude for this highly dynamic parameter, and rough estimates are the rule rather than the exception because -

1. Estimations of minute openings and porosities of building materials are extremely difficult.
2. Nonlinear effects of wind and inside-outside temperature differentials are not additive and are difficult to average.
3. Human activity in the homes is extremely variable and unquantitated.

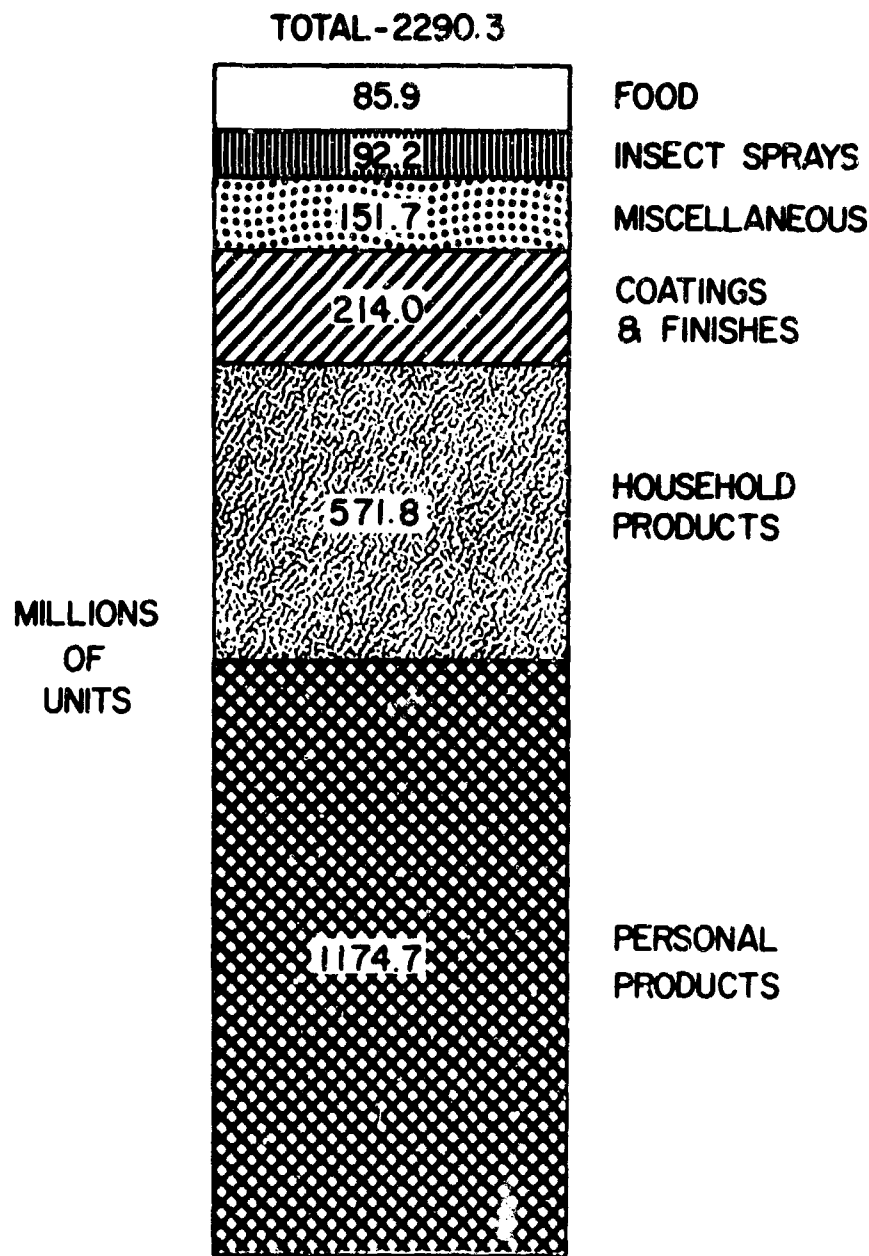
Measurements of infiltration rates are difficult and have been done accurately in only a few occupied homes. Such measurements involve the internal or external release of a tracer gas which behaves like air and can be measured in small quantities. In most cases where this has been done (Coblentz, 1963; Jordan, 1963; Tamura, 1964) infiltration rates seldom get above one air change per hour - less than half that amount is common. In the newer homes, where attempts to eliminate infiltration have been made, 0.2 air change per hour is common; and with additional efforts on the part of the householder to seal up all openings and avoid undue entrance and exit, this figure can readily be reduced by a factor of ten.

#### INCREASING CONTAMINATION OF THE HOME ATMOSPHERE

Reduction of infiltration rates is by no means the only factor influencing inside air contamination - our newly chemically enhanced lives testify to this fact. A quick perusal of the excellent reference volume, Clinical Toxicology of Commercial Products (Gleason et al, 1969) will astound the uninitiated with the ponderous number of chemicals of significant or unknown toxicities that may be found in the home. Many of these compounds are used with great caution in industry. Some of them - the new "enzyme cleaners", for example - are possibly hazardous, but show no indication of caution on their labels because of the lack of knowledge about their biological effects.

For this discussion, I chose as an example just one class of potentially hazardous materials - those dispensed in aerosol cans. Aerosols are used to dispense virtually every type of cleaning, cosmetic, hobby, and food material used in the home. The growth of this industry has been astounding. The journal, Aerosol Age, has shown a production growth from 500 million units in 1958 to over 2.29 billion units last year (Chemical Specialties Mfrs. Association, 1969). Figure 2 depicts this production, with over 1 billion units in personal products and 0.5 billion units in household products accounting for the majority. The largest volumes of aerosol products produced

in the United States in 1968 were hair sprays, personal and hygienic deodorants and anti-perspirants, starches and laundry products, finishes, and shaving lathers. The production growth rate of these preparations is expected to exceed 5 percent over the next few years.



A-991222

Figure 2. AEROSOL AND PRESSURIZED PRODUCTS PRODUCTION - UNITED STATES 1968



Modern technology has allowed the industry to further extend itself into the field of powdered aerosol products. These now include lubricants (graphite, molybdenum bisulfide, and Teflon), spot removers, insecticides, and foot powders. Numerous aerosol cosmetic powders are also available (Kubler, 1969). This innovation is expected to grow rapidly.

In addition to the "active ingredients", the propellant compound presents a hazard which deserves much attention. There are a number of propellents in use, but I will confine my remarks to the Freon compounds\* - mainly trichloromonofluoromethane, dichlorodifluoromethane, and 1, 2-dichloro-1, 1, 2, 2-tetrafluoroethane (Freons 11, 12, and 114). My concern is obviously not with toxicity of these chemicals themselves, but with their thermal and, to a limited extent, chemical decomposition products. Under a wide range of temperatures a number of toxic materials are produced. These include hydrogen fluoride, hydrogen chloride, carbonyl compounds, and other halogenated hydrocarbons, as well as free chlorine and fluorine. Water vapor and many metals tend to enhance the formation of decomposition products; temperatures as low as 130 F have elicited the production of these compounds (Church, 1961 and DuPont, n.d.). I must emphasize that the decomposition products resulting from these and other halogenated hydrocarbons are by no means completely defined.

Household heating and cooking equipment operates well within the temperature range for decomposition of these chemicals. The electric heating elements in furnaces, which often reach surface temperatures of 1500 - 2000 F, are within the closed environment system in the house. Corrosion of these elements and ducts has occurred as a result of chemical air contamination. Freons 11, 12, and 114 were shown to cause corrosion in heat exchangers in air concentrations as low as 1 ppm (Hama, 1965). However, with fossil fueled heating units we are not immediately concerned because the products of combustion are vented out of the system through a flue rather than back into the living space. Corrosion of the human system has not been investigated.

The average woman may use from 1/2 to 2 oz of hair spray per hair arrangement. In a 9000-cu-ft house, a peak concentration of 20 ppm of the Freon alone could be reached. Using a figure of 230 million lb of household propellents consumed in 62 million homes, it can be estimated that the average 9000-cu-ft house with an air-change rate of 0.2 per house would have a Freon concentration of 0.8 ppm at all times. Conversion of these figures to some of the more potent thermal decomposition products should give rise to legitimate concern for the health of individuals in the home on even an acute exposure basis.

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\*Freon - E. I. duPont de Nemours & Co., Inc.

It seems that long-term, low-level exposure due to the extensive use and leakage of these household products in the indoor environment would be of even more concern. The main point is that we know virtually nothing about the degree of overall chemical exposure in the home, let alone its possible biological effects. We do have some record of allergic or sensitive individuals responding drastically to indoor air pollution (Randolph, 1962), but we have little knowledge of the magnitude of this damage to the health and well-being of a population. Only recently have relatively few individuals raised the specter of indoor air pollution.

### AN EXPERIMENT IN ENVIRONMENTAL ANALYSIS AND CONTROL

In the previously mentioned study being conducted by the Institute of Gas Technology we have the unique opportunity to study in detail the indoor environment of two occupied test homes. These adjacent homes, precisely alike in all construction and furnishing details, are occupied by two similar test families. (Figure 3 depicts these homes and a nearby data collection enclosure.) A number of indoor environmental control systems were tested in these homes; both indoor and outdoor conditions are exhaustively monitored and controlled by a process-control computer which collects and records over 18,000 data points per day without interfering with normal family life (figure 4). We are currently measuring:

1. Indoor temperatures and humidities throughout the homes
2. Outdoor weather conditions, including solar radiation
3. Appliance-use patterns and energy consumption
4. Door and vent openings
5. Humidifier operation
6. Furnace and air-conditioner performance
7. Dust concentration and gaseous contamination
8. Infiltration



Figure 3. ENVIRONMENTAL TEST HOMES



Figure 4. DATA COLLECTION SYSTEM

Provision has also been made for real-time inputs to the data collection system from the test families regarding their responses to various environmental parameters found in the home.

Considerable time and effort have gone into developing the instrumentation and into obtaining base-line performance values for these homes. We needed to be sure that we could correlate parameters such as outdoor wind velocity, and heat loss from the house. But most important, we wanted to assure ourselves that the elusive infiltration factor could be accurately determined and accounted for. We have accomplished these goals and are in the process of moving on to more detailed analyses of the indoor atmosphere. We know, for instance, that at low wind velocities and at small inside-outside temperature differentials, these homes have an average infiltration rate of 0.1 air change per hour. This figure is common to most newly constructed homes where no special provisions for abolishing infiltration were made.

We have, moreover, seen the results of the use of aerosol sprays and know that 100 ppm of halogenated hydrocarbons in the indoor environment are not unusual. In fact, we were forced to abandon the use of one of these materials as a tracer gas for infiltration studies, largely because of the irregular ambient concentrations in the homes. Other trace constituents of the atmosphere were measured and await further verification before it is possible to make a definitive and meaningful report.

We plan to continue these studies of the indoor atmospheric environment on a much broader scale and hope to measure the effects of various control devices on contaminant levels. Future plans include the measurement of airborne microbiological contaminants, since it was shown (Elkins, 1969) that this is a highly significant factor in the spread of diseases in the home. We also plan to investigate the benefits to the indoor atmosphere of a positive-pressure, fresh air system.

### SUMMARY

It is apparent from existing literature and the relatively limited studies done in our own laboratories that the problem of contamination in the home will increase. We say this because of the increasing home use of potentially harmful materials and because of the definite tendency toward making the home environment a closed system. The task of defining the degree of contamination is only surpassed by the more monumental problem of ascertaining the subtle biological effects which may be caused by indoor air pollution; but we have seen that this problem is common to the evaluation of many open and closed systems.

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## DIFFERENTIAL SEMIMICRO METHOD FOR BLOOD CATECHOLAMINES

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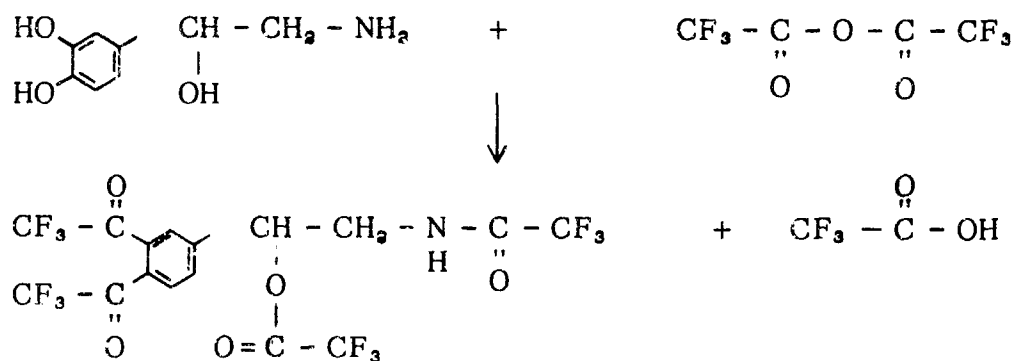
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Any toxicological investigation is dependent upon precise and accurate methods of chemical analysis. For many years, methods have been known for the *in vitro* and *in vivo* measurement of many compounds of biological importance. For the most part, however, these compounds are found in sufficiently high concentrations so that they do not require extremely sensitive methods of detection. On the other hand, there is an increasing interest in a number of chemicals of biological importance that are found in extremely low concentrations in blood, urine or tissue. In order to gain information concerning these compounds, new, extremely sensitive methods must be developed for their measurement. Such a group of compounds includes the biologically important catecholamines epinephrine, norepinephrine, and their metabolites. Tissue and urine measurement of these compounds has been possible for many years using fluorometric techniques; however, these methods are generally difficult to use in blood analysis for a number of reasons. First, and probably most important, are the interference problems that often occur with fluorometric methods. When working with blood, where there is a combination of very low catecholamine concentration and high concentration of other materials, particularly protein, interference and quenching phenomena lead to problems of precision, accuracy and sensitivity. Secondly, since the normal levels of epinephrine or norepinephrine found in the blood stream of humans average between 60 and 500 picograms per milliliter of plasma (White, Handler, Smith, Stetton, 1959), relatively large blood samples must be extracted in order to obtain sufficient quantities of the catecholamines for measurement by fluorometry. When using small laboratory animals, sufficiently large samples are almost impossible to obtain without terminal bleeding. It is therefore obvious that a new approach is necessary if these compounds are to be measured at the level at which they occur in the blood and realistic sample volumes are to be used.

For some time in our laboratory we have been using methods of analysis based on electron capture gas chromatography. We have developed a method of analysis for ionic beryllium in biological systems utilizing a gas chromatographic approach and for this reason we have attempted to apply a similar method to the analysis of the catecholamines. Since the chemical and physical properties of the catecholamines make them unsuitable for direct gas chromatography, we must first find suitable

derivatives for use in this type analysis. If we look at the chemical structure of catecholamines of biological importance, we find that they have a number of reactive groups which may possibly be derivatized to form a volatile compound that is amenable to detection using the electron capture detector. All of the compounds contain an amine group and at least two hydroxyl groups which are quite reactive. The literature reveals considerable work on gas chromatography of catecholamines. Horning et al (1964, 1966, 1968) have worked with trimethyl silyl ether and acetate derivatives while Landowne and Lipsky (1963) have done some work on monochloroacetates. These derivatives form quantitatively and are sufficiently volatile to allow chromatography of the catecholamines, however, none of them have significant halogen in the molecule and are, consequently, relatively insensitive to electron capture detection. On the other hand, Clarke et al (1966) at Fordham have worked with trifluoroacetate derivatives and found that these derivatives possess sufficient sensitivity to allow as little as 100 picograms of catecholamines to be measured. Although Clarke (1967) was successful in measuring one catecholamine, dopamine, in urine, he experienced some difficulty in separating the five catecholamines on any one chromatographic column. This fact led us to investigate the use of a number of different column materials in attempting to separate these derivatives.

The trifluoroacetate derivative can be easily formed at room temperature using a 20% solution of trifluoroacetic anhydride in acetonitrile. Reaction is immediate and quantitative with all five of the commonly occurring catecholamines as illustrated below. The by-product, trifluoroacetic acid,



can be removed rapidly by concentrating the reaction mixture under dry nitrogen. We investigated several different stationary phases of low polarity such as OV-25, SE-52, SE-30, and OV-1 but experienced the same problems of inefficiency that were found by Clarke using QF1 as a stationary phase. More polar phases, however, overcome some of these difficulties. EGSP-Z, an ethylene glycol succinate polymer, was found to separate 4 of the 5 catecholamine trifluoroacetate derivatives. OV-210, which is a methyl silicone form of QF1, would also separate 4 of the 5 catecholamines but they were 4 different catecholamines than those separated by EGSP-Z. Instead of investigating other polar phases we decided to test various mixtures of these two. Figure 1 shows a chromatograph of the five catecholamine trifluoroacetate derivatives using a 6 ft glass column containing a 1:1 mixture of the two phases. A hydrogen flame

ionization detector was used to avoid concentrating our samples to remove unwanted reaction products. There is a slight overlap between norepinephrine and dopamine. However, by varying the ratio of stationary phases, this can be avoided. For instance if a 60/40 column is used, dopamine and norepinephrine separate cleanly; however the dopamine-metanephrine separation is less than baseline.

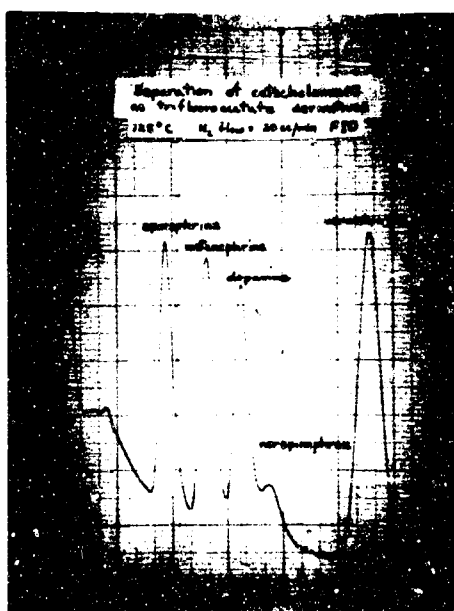


Figure 1 CHROMATOGRAPH OF A STANDARD SOLUTION CONTAINING FIVE CATECHOLAMINES AS TFA DERIVATIVES USING 1% EGSP-Z: OV210 60:40 w/w AS A STATIONARY PHASE. Gaschrom Q 60/80 mesh used as the solid support.

Since we had developed an analytical approach for measuring catecholamines in standard aqueous solution, we then attempted to apply this to the measurement of one of these amines (epinephrine) in spiked blood samples. This presents the problem of quantitative extraction and prevention of degradation during the extraction procedure. First we tried direct reaction of trifluoroacetic anhydride with freeze-dried samples which were spiked before freeze drying with epinephrine. Using this method, recoveries of the epinephrine were acceptable but there were many extraneous peaks in the chromatograms. With the acidic butanol extraction method used by Maikel (1968), we obtained cleaner samples but less quantitative results. Since protein-free filtrates have been used extensively for removing small molecules from biological samples, trichloroacetic acid precipitation followed by direct reaction of the freeze-dried filtrate was



attempted. Trichloroacetic acid is soluble in the reaction solvent and cannot be easily removed and consequently interferes with chromatography. The analogous fluorine acid, however, can be easily removed by freeze drying due to its high vapor pressure. Its action as a precipitating agent is very similar to trichloroacetic acid. If the protein-free filtrate obtained is freeze-dried, both the water and the trifluoroacetic acid are removed and the end result is a clean sample. Recoveries from spiked samples range from 80 - 100% using this method of extraction. Chromatographs of two samples are shown in figure 2.

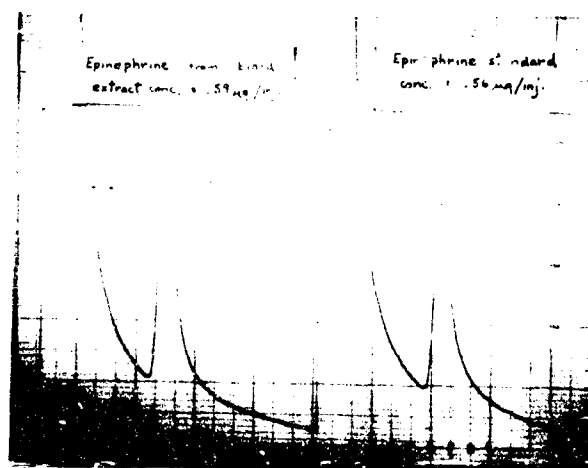


Figure 2. COMPARISON OF A CHROMATOGRAPH OF AN EPINEPHRINE TRIFLUOROACETATE DERIVATIVE IN A STANDARD ACETONITRILE SOLUTION WITH THE SAME COMPOUND EXTRACTED FROM WHOLE BLOOD SPIKED WITH 2 MG/ML EPINEPHRINE. Column and conditions are the same as in figure 1.

The chromatograph on the right is a weighed amount of epinephrine reacted with trifluoroacetic anhydride to form the derivative and then chromatographed. The other is a blood sample containing two milligrams per milliliter epinephrine, extracted and derivatized by the method described previously. The peak in this chromatograph corresponds to .59 micrograms of epinephrine as opposed to .56 micrograms of epinephrine/injection in the standard. As can be seen, both extraction and the deriva-

tive formation appear quantitative. While a concentration of two milligrams per milliliter of blood is several orders of magnitude higher than the normal baseline level of epinephrine, the chromatograph still serves to show the feasibility of the approach. Furthermore, this is far from the practical sensitivity of the method at this stage of development. By using a combination of concentration steps and the more sensitive electron capture detector instead of the  $H_2$  flame ionization detector, concentrations on the order of .01  $\mu\text{g/ml}$  epinephrine or norepinephrine in blood samples of 1 ml might be realized. This sensitivity, however, is still insufficient to allow measurement of catecholamines in normal human blood or plasma, particularly if we are restricted to small (1 ml) sample volumes. At this time we are pursuing two solutions to this problem which may either separately or together provide the required sensitivity. The first and most obvious solution is the preparation of a new derivative which is more amenable to electron capture detection. In a recent paper, Angaard and Sedvall (1969) have shown that heptafluorobutyrate derivatives of several catecholamine metabolites including metanephrine and normetanephrine are much more electron capturing than the trifluoroacetates. cursory studies in our laboratory have indicated that this is indeed also true for epinephrine and norepinephrine. With these derivatives, levels as low as 10 picograms epinephrine per injection produce quantifiable peaks. This is almost an order of magnitude less than can be detected of the corresponding trifluoroacetate derivative. A second possibility to increase the sensitivity of the method would be to increase the sample size injected into the chromatograph. While solvent peak interference becomes a problem for sample sizes greater than 5 microliters, several years ago Glowall Industries developed a device whereby large (.5 ml) samples could be impregnated on steel wool pads and the solvent removed by evaporation. Pads containing the sample were then directly dropped into the heated injection port of the chromatograph. We are presently working on a modified version of this device and have successfully used it to inject the material contained in up to 0.2 ml of solvent with no solvent interference whatsoever. While much more work will be required, it appears that these two modifications to the method described will provide adequate sensitivity to allow measurements at realistic biological levels.

In summary, the details of our present method are as follows:

1. To 1 ml whole blood, add 3 ml aqueous 10% trifluoroacetic acid and agitate for 30 seconds.
2. Centrifuge for 30 minutes to precipitate blood proteins.
3. Withdraw a 1 ml aliquot of the supernatant and freeze-dry.
4. To the freeze-drying vial, add 1 ml 20% trifluoroacetic anhydride in acetonitrile.
5. Chromatograph 1 microliter sample using 6 ft columns packed with 1% EGSP-Z/OV210 3/2 W/W on 60/80 mesh gas Chrom Q, 125 C column temperature, 140 C injector temperature, 190 C detector temperature, 20 cc/min nitrogen flow.

With the modifications I have suggested, it is hoped that this will provide the long sought after differential semi-micro method of analysis of blood catecholamines.

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EVALUATION OF FIELD INSTRUMENTATION FOR  $N_2O_4$   
AND MONOMETHYLHYDRAZINE

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INTRODUCTION

During the first of our conferences, a discussion ensued subsequent to Conkle's paper (1965) on contaminant analysis, concerning the reliability of the "black box" vs classical chemical methods. The recorded consensus attested to the dissatisfaction, to one degree or another, with totally instrumented approaches. The purpose of this paper, presented four years after this brief discussion, is to describe an essentially identical situation - in this case the analytical monitoring dilemma involving the hypergolic missile propellant systems of monomethylhydrazine (MMH) and nitrogen dioxide/nitrogen tetroxide. In an attempt to convey the totality of the problem, we will dwell on several subjects, i.e., evaluations of prototype and developed instruments and colorimetric dosimeters, commercially available portable detector, and recently obtained data on a specific field experience. An examination of previous considerations and attempts to develop detection systems characterized by operational requirements should prove enlightening.

Although a wide diversity of chemical types might be included in this general discussion, we may limit our interest to those propellants currently in use or those most likely to be utilized in the foreseeable future. The former include the hydrazine family of fuels and the oxidizer  $N_2O_4/NO_2$ , while the latter consists of inorganic halogens, interhalogens, NF compounds such as nitrogen trifluoride and tetrafluorohydrazine, chlorine trifluoride, oxygen difluoride, and chlorine pentafluoride. Also of interest are the propellant mixtures incorporating the light metals aluminum and beryllium.

The development of appropriate devices and techniques for the detection of missile propellant vapors meets its first obstacle in the definition of requirements. We may consider two distinct categories of needs: 1) the leak detector that is designed to respond to the minutest concentration within the state-of-the-art capability to ascertain the status of the weapon system; and 2) the device that monitors the environment to provide protection/warning for workers with respect to safe limits as defined by in-

dustrial hygiene dictates. Obviously both the design goals and selection of the operational principle must be guided by the specific application of the device. Those considerations that influence approach and design include sensitivity, accuracy, dynamic range, portability, power consumption, duration and intent of use, interferences, reliability and cost. Further complication occurs when field requirements, and hence logistic problems, are considered.

In order to delimit the scope of this discussion, we will arbitrarily reject the leak detector concept and address ourselves to the research and development efforts associated with devices designed to provide warning of a hazardous work environment. Certainly an instrument that can operate continuously and quantitatively detect propellant vapors in the fractional part per million (ppm) range is a desired goal and would offer an adequate solution to both problems. However, such an instrument is difficult to obtain for laboratory applications and is far from a reality for field use. Suffice it to say that leak detection requirements will have to be satisfied with the performance of industrial hygiene instruments whose sensitivity and duration of performance requirements are considerably less stringent.

To ascertain if nonhazardous conditions (relative to toxicity) exist during the performance of testing or operational procedures associated with missile systems, two approaches have been considered: 1) the utilization of colorimetric methods in which the development of a characteristic color on a supporting surface provides a quantitative estimate of vapor concentration; and 2) the application of selected physico-chemical principles in conjunction with electronic signal processing to provide a direct measurement of environmental concentration. Two goals were characteristic of both of these programs: sensitivity in the range of the established industrial hygiene limits, i. e., the Threshold Limit Value (TLV) and the Emergency Exposure Limit (EEL), and specificity (or lack of response to potential interfering substances).

Colorimetric techniques for the detection of a wide variety of toxic substances have been included in the armamentarium of the industrial hygienist for some time. In essence, air samples are passed through a glass tube containing the reagent adsorbed on a supporting material such as silica gel or on a piece of filter paper placed in a holding device. A metered amount of air is sampled by virtue of a calibrated hand pump and a limiting orifice. The intensity of the developed color or the length of the colored portions of the column is compared to standards for a quantitative estimate of the concentration in air. Sensitivities may be quite low, e. g., arsine may be reliably detected at 0.025 ppm. In general the usable response ranges encompass the TLV. For the propellants of interest in this report, i. e.,  $\text{NO}_2$  and monomethylhydrazine, commercially available detector tubes (Draeger Tubes Ch318 and Ch300) were shown to respond to 1 ppm MMH and 5 ppm  $\text{NO}_2$  respectively (Luffly et al, 1968). However, extremes of temperature and high humidity significantly affected the quantitative response.

These types of colorimetric detectors require the user to participate actively in the measuring or monitoring procedure. In addition, the information obtained represents a single point in time as opposed to a continuous or integrating record. Under certain work situations such as occur in missile silos or launch control centers, these characteristics could prove unacceptable. To obviate these undesirable performance characteristics, colorimetric film badges were developed that operate by passive diffusion and are integrative, i.e., they exhibit an increasing color intensity with increasing concentration and/or time. Thus it is possible to obtain an approximation of the extent of exposure in terms of a concentration x time (CT) value. Plantz (1967) developed a dosimeter for hydrazine fuels using the reagent bindone (  $\Delta^1,2$  - biindan) -1',3,3'-trione) adsorbed on silica gel. This system provides a color response over the CT range of 65-1800 ppm-min depending upon the particular hydrazine fuel. Arnold and Rakowski (1968) presented a detailed description of the chemistry of the bindone - hydrazine reaction and an evaluation of the dosimeter response, indicating performance deficiencies, recommendations to rectify these deficiencies, and proper utilization of the device in the field. Rakowski (1969) compared the capabilities of several commercially available colorimetric detectors for hydrazines and  $\text{NO}_2$ , noting those that may be more amenable to Air Force needs. Rakowski (1969) also developed dosimeters for  $\text{NO}_2$  using diphenylamine on silica gel that gave well-defined color differentiation at CT values of 50, 100, and 300 ppm-min. Diamond (1968) evaluated these sensor strips and observed a reasonable agreement of the response with the color standards formulated by Rakowski.

All of these dosimeters exhibit several undesirable features, primary of which is the lack of quantitative accuracy. However, if used judiciously they could provide adequate industrial hygiene information.

A device that utilizes both colorimetric and instrument approaches is the E41 alarm<sup>1</sup>, which was originally conceived as a field detector for chemical warfare agents. In essence, an air stream containing the agent is impinged on a tape impregnated with the reagent. The presence of the agent results in a colored spot that is observed by a photo detection system comprised of an exciter lamp and a cadmium sulfide photocell. A study conducted by Autonetics (1969) to determine the efficacy of this instrument for Minuteman III application indicated that a modified E41R3 alarm using dry fiberglass tape impregnated with alternating spots of N-phenyl anthranilic acid and p-dimethylaminobenzaldehyde (for  $\text{NO}_2$  and MMH respectively) detected the propellants at the TLV concentrations. Response times were acceptable, and the system performed adequately between the relative humidity (RH) range of 0-100% and temperature range of 0-38 C (32-100 F). However, certain deficiencies were noted and Autonetics presented recommendations for a developmental effort for the redesign of the E41 alarm to fulfill Minuteman requirements. The E41 alarm was tested by Prager (1968b) for use as a shipboard detector and found to perform satisfactorily for MMH detection.

<sup>1</sup>U.S. Army Training Manual TM3-6665-210-12, Alarm V-G Agent, Automatic Field, E-41A3

Prager (1968a) also studied the response of the E41 to chlorine trifluoride (CTF). Using the alarm in its initial configuration, i. e., addition of the reagent O-dianisidine in solution to the tape, he observed a response to 0.12 ppm CTF in less than 30 seconds and to 0.48 ppm in 10 seconds.

The search for appropriate physico-chemical methods for propellant vapor detection has yielded several interesting and potentially efficacious approaches. Annand et al (1963) evaluated a variety of physical and chemical phenomena, shown in table I, and selected three methods which appeared to exhibit desirable characteristics for further study. Their experimental investigations on surface conductivity of semiconductors, ionization by radio frequency excited helium, and halogen-enhanced flame ionization indicated that ppm levels of propellants could be detected by these techniques. These studies were only exploratory in nature and were not designed to result in instrument development.

TABLE I  
POTENTIAL TECHNIQUES FOR PROPELLANT DETECTION

<u>Technique</u>	<u>Sensitivity</u>
<u>Surface Adsorption on Metals</u>	
1. Surface Potential	Very Good
2. Capacity of Vibrating Condenser	Very Good
3. Resistance of Thin Films	Good
4. Ellipticity of Polarized Light Reflected from Thin Films	Good
5. Field Emission Microscopy	Best
<u>Surface Adsorption on Semiconductors</u>	
6. Strobe Measurement of Charge Carrier Generation	Very Good
7. Surface Conductivity of Thin Films	Very Good
<u>Ionization Processes</u>	
8. Halogen Enhanced Flame Ionization	Good
9. Ionization by rf Excited Helium	Very Good
10. Field Ionization	Best
<u>Dielectric and Electromagnetic Phenomena</u>	
11. Fluorescence Quenching	Good
12. Microwave Interferometry	Good
<u>Miscellaneous</u>	
13. Corrosion Rate Effects	Good
14. Surface Tension Effects	Poor

Modified from Annand et al (1963)

Brousaides et al (1961) studied the feasibility of activation analysis and inverse radioactive tracer techniques for propellant detection. Using  $^{85}\text{Kr}$  quinol clathrates, they showed that  $\text{NO}_2$ , hydrazine, and unsymmetrical dimethylhydrazine (UDMH) could be detected and recommended an instrumented approach using this principle. They also discussed a neutron production and detection method for BeO measurement.

An amperometric method was investigated by Poulos (1961) for  $\text{N}_2\text{O}_4$ ,  $\text{O}_3$ ,  $\text{N}_2\text{F}_4$  and UDMH detection. He adapted a prototype borane monitor using Pt-Pt or Pt - Ag/AgCl cells and could measure the compounds of interest at the 0.1-10 ppm level. Roth (1963) discussed the methods used at that time for liquid propellant monitoring and considered others that might be applicable (catalytic combustion, thermal conductivity, depolarization, infrared absorption, ionization and colorimetric titration).

Prager and co-workers (1964, 1968a, 1968b) have investigated the applicability of several detection principles for various propellants of interest under laboratory and field (shipboard) conditions. The methods considered included colorimetry (E41), ionization chamber, electrochemical, gas chromatography with flame ionization and electron capture detectors, fluorescence quench, frustrated multiple internal reflection (FMIR) spectroscopy, and condensation nuclei counter. Prager's studies have indicated that the ionization chamber in a two-channel configuration is the best of these approaches for Navy requirements.

All of the principles discussed above suffer from the restrictions imposed upon any detection device that must operate as a point-source sensor, with the exception of infrared absorption. Using infrared scanning in an open path mode, it might be possible to negate the problems attendant with multiple sensors. Buscaglia and Wallack (1961) considered such an application and showed the feasibility of narrow absorption infrared (NAIR) instrumentation for  $\text{NO}_2$ , UDMH, and  $\text{N}_2\text{H}_4$  detection. In essence, the system is based upon nondispersive detection employing a microphone-type transducer for the conversion of thermal to electrical energy mediated by mechanical (gas pressure) means. Based upon this study, Freilino and co-workers (1963) designed and fabricated a prototype device that utilized a folded path up to 250 ft in length. These efforts indicated the advantages of area monitoring and the selectivity of IR approaches.

Field detection requirements for missile propellants were first established in 1960 with the advent of the Titan II Ballistic Missile System. It was considered necessary to incorporate permanently installed and portable monitoring equipment to fulfill system and safety requirements. The fixed detection system consisted of a commercially available ionization chamber while the portable device operated on the electrochemical principle. Both of these devices proved to be of little or no value due to instrument malfunction, lack of sensitivity and selectivity, and difficulty in operation (Russell, 1969). However, improved models of the electrochemical device became available and were evaluated in detail by Kleibenberg and Widman (1969). They showed that the Teledyne Olfatron<sup>®</sup> 6010 would respond to 2 ppm MMH, UDMH and  $\text{NO}_2$  under carefully controlled laboratory conditions. Concentrations lower than 2 ppm could not be reliably detected.



Increased emphasis on the need for MMH and  $\text{NO}_2$  monitoring devices was provided by the inception of the Minuteman III System. The detection requirements included portable operation on both 110V ac from the system and self contained battery pack at detection sensitivities of 1.0 ppm MMH and 5.0 ppm  $\text{NO}_2$ . Teledyne had developed a further modification of the Olfactron, designated Model 6110, and this device was considered for fulfilling the above system requirements. Changes in electronics, sampling rate, and sensor design suggested that significant improvements had been incorporated. To ascertain the reliability and capability of this instrument, we initiated a detailed evaluation of the most recent modifications.

## MATERIALS AND METHODS FOR OLFACTRON EVALUATION

### UDMH and MMH Procedures

The instrument evaluated is the Olfactron Portable Vapor Detector Model 6110 which is manufactured by the Control Systems Division of Teledyne Inc., El Segundo, California. It is a self-contained instrument consisting of four sections: the oxidizer and fuel transducers mounted in tandem in conjunction with a fan that provides the pneumatic sampling train; the solid state electronic signal processing section; the audio and visual alarms and single readout meter that can be switched for either channel; and the power supplies consisting of a 20-cell storage battery pack that provides an output of positive and negative 13 volts dc for eight hours operation and re-actifier power supplies for ac continuous operation and battery charging. A three-position range selector switch (X5, X1, and X.1) permits meter readings from 0-500 ppm for the oxidizer channel and from 0-100 ppm for the fuel channel. As recommended by the manufacturer, the Olfactron should be calibrated with the Teledyne Calibration chamber, Model 6190. This is a 10 liter teflon-lined aluminum chamber in which the transducer and fan may be placed for calibration. It is provided with external electrical fittings to enable the Olfactron to serve as the electronic and read-out source. However, the chemical stability of the hydrazine fuels is such that establishment of nominal concentrations under static conditions without means of verification can be seriously questioned. To avoid such potential problems we decided to utilize a dynamic system with continuous on-line monitoring of the fuel concentration by automated, instrumented means, as depicted in figure 1. In this system, MMH or UDMH was injected via a port into a 20 liter teflon bag which contained dry nitrogen and allowed to vaporize to establish a nominal concentration of approximately  $10^4$  ppm. The hydrazine fuel (in nitrogen) was pumped to the mixing chamber where it was diluted with room air that had been dried by passage through the silica gel canister. The fuel-laden air stream passed through a flowmeter and was then divided into three portions. One portion passed through a metering valve and a flowmeter to a Technicon Auto-Analyzer<sup>®</sup> where the hydrazine fuel concentration was measured continuously by the iodine reduction method essentially in the range of 10-50 ppm (Geiger, 1967). A second portion was drawn, by virtue of the contained pump, through another metering valve to the MSA Billion-Aire<sup>®</sup>. This device, which operates on the ionization chamber principle using trifluoroacetic acid vapor as the particulate-forming reagent, was used to monitor the propellant concentrations in the 0-10 ppm range. Calibration was accomplished with the Auto-Analyzer.

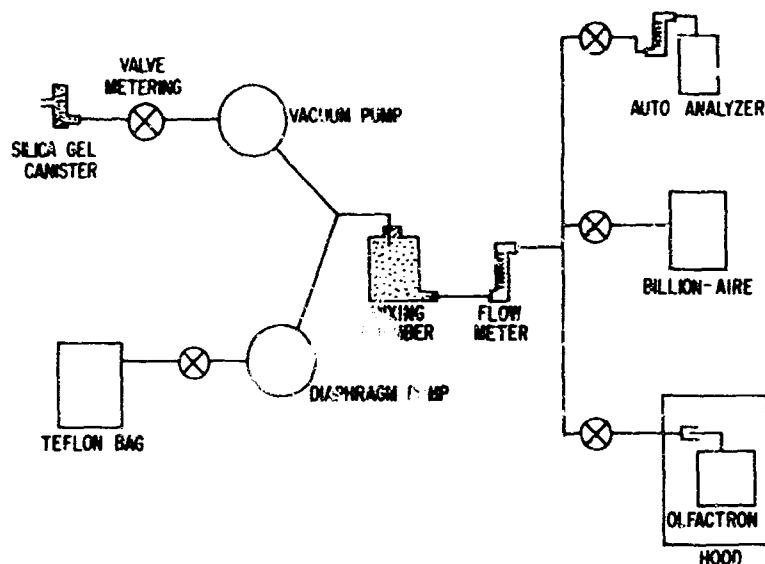


Figure 1. DYNAMIC FLOW SYSTEM

In all experiments both instruments were used simultaneously at all fuel concentrations; by diluting the fuel-containing air with room air via a meter valve and flowmeter, the Billion-Aire could monitor concentrations ranging from 0-50 ppm, while changing the normality of the iodine solution from 0.001 to 0.01 permitted using the Auto-Analyzer over this range. The third portion of the fuel-air stream entered an expansion chamber, located in a hood, consisting of a length of Tygon<sup>®</sup> tubing (2.5 x 7 cm) into which the probe of the Olfactron was placed. The flow rate at this point could be calculated by subtracting the sum of the flow rates through the analytical instruments from the total flow of the system. Thus it was possible to maintain and monitor with adequate precision and accuracy (Geiger, 1967) the desired fuel concentration in a dynamic system during the entire experimental period.

The MMH and UDMH used were propellant grade (99.4% purity) and were obtained from Olin Matheson Chemical Corporation and Matheson Coleman & Bell, respectively.

Data were obtained in the following manner; prior knowledge of system flow rates was used to establish a nominal UDMH or MMH concentration. When the recorders indicated the system was passivated and equilibrium had been reached, fine adjustments of the flow rates were performed to obtain the particular concentration of interest. Upon reestablishment of a flat baseline, the Olfactron probe was placed in the expansion chamber and sampling initiated. Throughout the entire experiment, the Olfactron was operated continuously in the ac, i.e. line current, mode. At the initiation of each phase of the evaluation, the transducer was calibrated by adjustment of the amplifier to produce the meter reading equivalent to the known fuel concentration.

Transducer response was measured by observing the meter at selected time intervals. Recovery times were determined by removal of the Olfactron probe from the expansion chamber and permitting room air to flow through the pneumatic system. Determinations at each fuel concentration were performed in triplicate; prior to each observation, the transducer was equilibrated, i. e. a stable meter reading was obtained at 0 ppm or the meter was adjusted to this point.

### NO<sub>2</sub>/N<sub>2</sub>O<sub>4</sub> Procedures

At dilute concentrations at room temperature the monomer/dimer mixture of NO<sub>2</sub> and N<sub>2</sub>O<sub>4</sub> reaches an equilibrium in favor of NO<sub>2</sub>. Since NO<sub>2</sub> is stable in air (Carberry, 1959; Dekker et al, 1959), we did not feel it necessary to monitor an absolute concentration but rather determine the linearity of the transducer response with respect to nominal but quantitatively proportional concentrations.

Our studies of the oxidizer transducer were conducted with the Olfactron calibration chamber. The transducer was calibrated by establishing a 50 ppm concentration of NO<sub>2</sub> in the chamber by injection of 293  $\mu$ l of NO<sub>2</sub> vapor at 23 C. The volume of NO<sub>2</sub> required was calculated as described in the Olfactron manual using the relationship  $V_g = \frac{NV_c}{eP}$

where  $V_g$  = volume of gas,  $N$  = ppm,  $V_c$  = volume of the chamber,  $e$  = temperature correction factor, and  $P$  = pressure in atmospheres. All subsequent data were obtained by injection of the calculated volume of NO<sub>2</sub> vapor to establish the desired chamber concentration. Each experimental point was replicated at least three times. The NO<sub>2</sub>/N<sub>2</sub>O<sub>4</sub> used in this study was obtained from the Mateson Company in lecture bottles.

The studies concerning transducer response to humidity were performed with old and new transducers placed in tandem in the Olfactron. Since the amplifier circuitry is the same for both oxidizer and fuel channels, it was possible to use each of the channels for both oxidizer transducers. The transducer responses were recorded on a dual-pen strip chart recorder. Both transducers were set arbitrarily at a meter reading of 10 ppm at the initiation of the experiment. The Olfactron probe was then connected to Mylar bags containing either dry air or saturated air as required.

## RESULTS AND DISCUSSION OF OLFACTRON EVALUATION

The primary objective of this effort was threefold: to determine the sensitivity and linearity of response to MMH; to ascertain the validity of MMH calibration using UDMH; and to determine the performance of the new NO<sub>2</sub> transducer. Our initial studies were conducted with UDMH since it is considerably more stable than MMH and it is necessary to determine differences in transducer response.

The representative data in table II are presented to show the excellent agreement in response for replicate observations. Transducer No. 2 was calibrated with 48 ppm UDMH and then exposed to successive concentrations of UDMH. The data points at the selected time intervals for a given exposure were in many instances identical.

TABLE II  
TRANSDUCER NO. 2 RESPONSE RATE TO UDMH

Seconds								
	5	10	20	30	45	60	120	Final
Run - 21.0								
1	19	21	23	24	24	24.5	26	26
2	18	21	22	22.5	23.5	24	25.5	26
3	19	21	22	23	23	23.5	24.5	26
Run - 9.7								
1	6	7.5	8.5	9.0	9.4	9.6	10.0	10.4
2	6.5	8.0	8.7	9.2	9.5	9.7	10.0	10.3
3	7.0	8.2	9.0	9.3	9.6	9.7	10.0	10.4
Run - 3.0								
1	1.2	1.8	2.2	2.3	2.5	2.6	2.9	3.3
2	1.4	1.8	2.2	2.4	2.6	2.7	3.0	3.2
3	1.4	1.8	2.3	2.6	2.7	2.8	3.0	3.3

Numbers in the Table are Olfactron meter readings in ppm

The time for maximal response varied from approximately 2 to 15 minutes. With the exception of the 0.5 ppm exposure, the final readings, shown in figure 2, did not significantly exceed the 2 minute response. These data indicate a reasonably linear response to UDMH. The response of the instrument to concentrations of UDMH ranging from 0.5 to 56.5 ppm, plotted in terms of percent response is shown in figure 2. This response varies from approximately 90% to 124% of the actual exposure concentration without any apparent relationship of the concentration to final response. The recovery for the 21 ppm exposure, the first to be accomplished, was significantly greater than 100% because of excess electrolyte on the sensor surface which was removed for subsequent exposures. Although these response curves do not cluster around the 100% level as closely as we would prefer, the data do suggest the Olfactron can provide an indication of UDMH concentration.

The recovery data for this experiment presented in table III suggest an inverse relation of exposure concentration to recovery time. The trend remained constant throughout our studies as shown in table IV and V, and figure 7. We attribute this observation to the fact that as the exposure concentration decreases, the 10 or 20% final recovery to 0 baseline represents smaller ppm meter readings. At the higher amplifier gains required for the lower concentrations, these smaller fractions are more apparent as the system depassivates and approaches equilibrium.

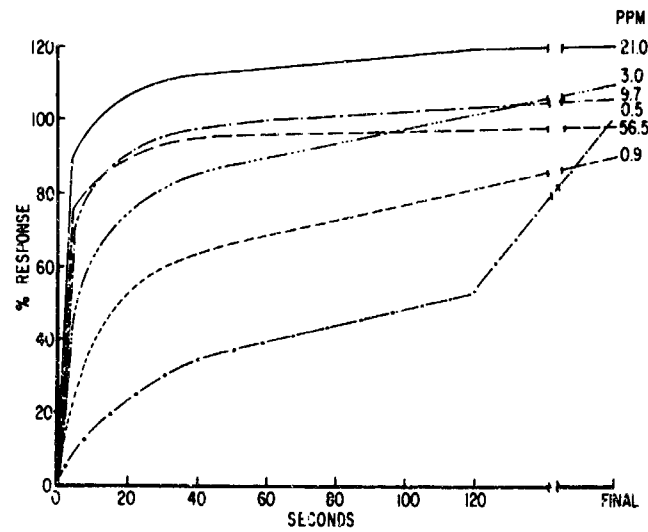


Figure 2. TRANSDUCER NO. 2 RESPONSE TO UDMH

TABLE III

RECOVERY OF TRANSDUCER NO. 2 AFTER EXPOSURE TO UDMH

UDMH, ppm	Recovery Time in Seconds	
	80%	90%
56.5	21	113
21.0	8	36
9.7	10	42
3.0	24	91
0.9	44	85
0.5	570	--

Transducer Calibrated with UDMH

TABLE IV  
RECOVERY OF TRANSDUCER NO. 3 AFTER EXPOSURE TO MMH

MMH, ppm	Recovery Time in Seconds	
	80%	90%
55.0	5	27
34.6	17	131

Transducer Calibrated with 24 ppm UDMH to Read 35.5 ppm MMH

TABLE V  
RECOVERY OF TRANSDUCER NO. 3 AFTER EXPOSURE TO MMH

MMH, ppm	Recovery Time in Seconds	
	80%	90%
17.5	6	29
11.7	28	368-55
2.7	52	97
1.2	73-4	--

Transducer Calibrated with 51.5 ppm MMH

The next phase of our effort concerned the calibration of the transducer with UDMH for MMH monitoring. The amount of UDMH required to establish a desired concentration in the calibration chamber can be calculated from the relationship:

$$V_L = \frac{V_c K N \cdot 10^{-3}}{D}$$

where  $V_L$  = volume of UDMH in  $\mu$ l;  $V_c$  = volume of the chamber (10 liters);  $K$  = 2.46  $\mu$ g UDMH/liter of air/ppm;  $N$  = UDMH chamber concentration in ppm; and  $D$  = density of UDMH at 25 C (0.7861). If one were to calibrate with MMH, appropriate substitutions in the equation for MMH density and weight-ppm equivalent should produce the required volume for a given concentration, provided all the MMH is vaporized. Because of the problems attendant with field calibration using MMH, Teledyne experimentally determined appropriate conversion tables for calibration with UDMH. For example, 1.6  $\mu$ l UDMH is required to establish a 50 ppm chamber concentration of this fuel while a value of 1.08  $\mu$ l UDMH is given in the Olfactron Service and Calibration Manual as producing a 50 ppm MMH equivalent. Using this ratio of 1.6/1.08, we exposed fuel transducer No. 3 to 24 ppm UDMH as measured in our dynamic system and spanned the instrument to read 35.5 ppm MMH. The responses of this transducer to 24.6 and 55 ppm MMH are shown in figure 3. It is quite apparent that the final values of 60 and 56% represent

a significant reduction in response. We conclude that this was due to the use of a conversion factor that was erroneously derived. Our data suggest that the transducer responds differently when exposed to MMH and to UDMH. Recovery times for MMH are similar to UDMH as indicated by the data obtained for this phase of the study shown in table IV.

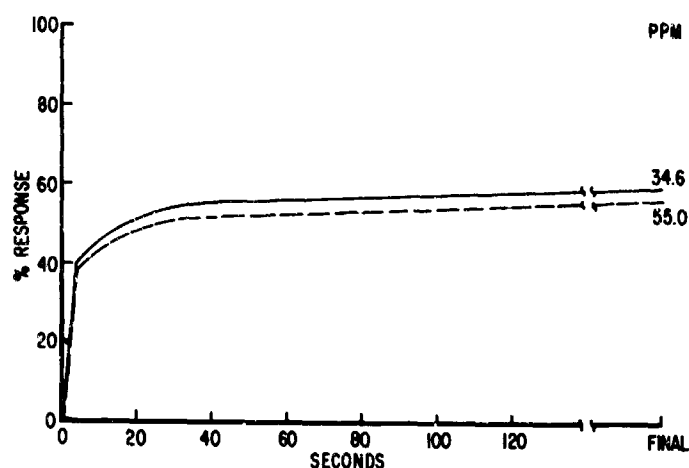


Figure 3. TRANSDUCER NO. 3 RESPONSE TO MMH CALIBRATED WITH 24 PPM UDMH TO READ 35.5 PPM MMH.

Since the quantitative response to MMH could not be determined when the transducer was recalibrated with UDMH, transducer No. 3 was recalibrated with MMH at a concentration of 51.5 ppm. A series of four exposures of MMH concentrations ranging from 1.2 to 17.5 ppm was then performed. The results of this study are shown in figure 4 and table V. The observed responses ranged from 16 to 90%; in this case, the data suggest a direct relation between exposure concentration and percent response. However, the sequence of exposure was from highest to lowest concentration which might have been a contributing factor. The recovery data presented in table V indicate both the increased recovery time with decreasing concentration, as well as significantly variable data due, we surmise, to the greater instability of the MMH molecule.

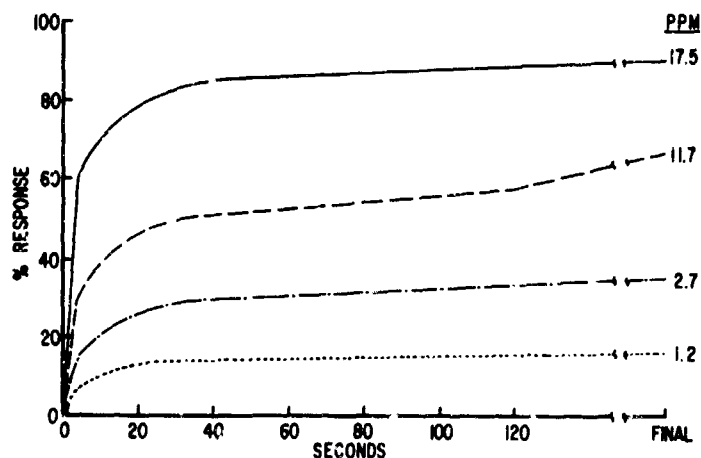


Figure 4. TRANSDUCER NO. 3 RESPONSE TO MMH  
CALIBRATED WITH 51.5 PPM MMH.

Our initial studies with the oxidizer transducer and  $\text{NO}_2$  indicated that a linear response occurred within the concentration range studied as shown in figure 5. However, a more detailed series of tests concerned with response times and completeness of response produced results similar to our observations with the fuel transducer. As can be observed in figure 6, the responses varied from 90 to 105 percent without any apparent relation to concentration or order of exposure. Although this range of responses is not as great as observed previously and certainly more closely approaches the desired goal of 100 percent, all of our observations with respect to overall response vary sufficiently to suggest that these transducers are characterized by what might be termed "fatigue". Another similarity concerning recovery time was also observed with  $\text{NO}_2$  as is depicted in figure 7.



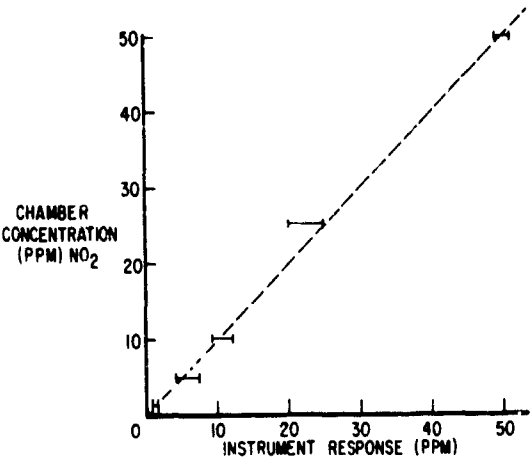


Figure 5. CALIBRATION CHAMBER WORKING CURVE FOR  $\text{NO}_2 \rightleftharpoons \text{N}_2\text{O}_4$

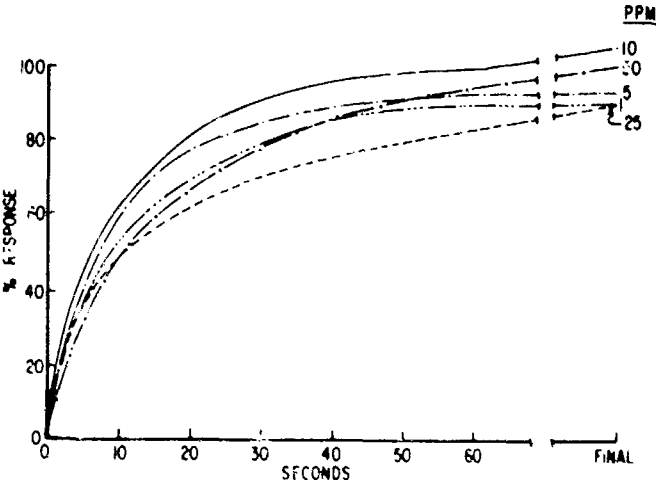
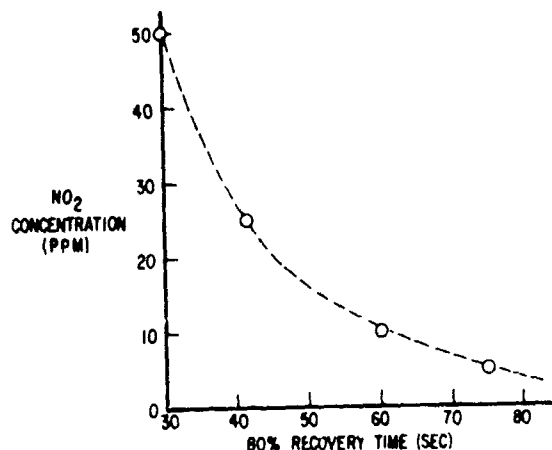


Figure 6. OXIDIZER TRANSDUCER RESPONSE TO  $\text{NO}_2$

Figure 7. RECOVERY TIME AFTER NO<sub>2</sub> EXPOSURE

To ascertain if a fatigue phenomenon did, in fact, exist we placed protector caps over the ends of transducer No. 3 and stored it for 48 hours. The transducer was then replaced in the Olfactron and exposed to 68 ppm MMH as determined with the Auto-Analyzer. For three successive exposures, meter readings of 67, 68, and 68 ppm were observed. Upon reevaluation of previous data, we considered the possibility of a concentration effect and therefore removed the transducer, recapped it, and stored it for an additional 48 hours. The transducer was again replaced in the instrument and exposed to 10, 13, and 13 ppm MMH (the Billion-Aire reading changed during this study but Olfactron readings were obtained simultaneously); the transducer response was 10, 14, and 14 ppm. As an additional determination, transducer No. 2 which had been used for the UDMH studies, was rechecked without recalibration. Upon exposure to 73 ppm UDMH (Auto-Analyzer) a reading of 70 ppm was obtained (96 percent response). Thus, the deviations of our data from 100 percent response, as shown particularly in figure 4, may be interpreted as resulting, at least in part, from fatigue of both fuel and oxidizer transducers to repeated exposures. Since these electrochemical devices are in essence fuel cells, it is conceivable that small amounts of fuel or oxidizer and/or their degradation products continue to exist within the milieu of the electrolyte that is in intimate contact with the solid state sensor surface. These substances can interfere, by an inhibition, with the normal oxidation or reduction process occurring within the cell. Total recovery, i.e., 100 percent response, can occur only after these substances have dissipated by depassivation or degradation to innocuous constituents. It is therefore mandatory, irrespective of the underlying mechanisms, that this characteristic of the Olfactron transducers be completely elucidated prior to any further consideration of this instrument for field use.

Brousaides' (AF Cambridge Research Laboratories, Hanscom Field, Personal Communication) studies on the older oxidizer transducer indicated that humidity exerts such a significant effect that the transducer responds in an almost linear fashion that can totally obscure its response to  $\text{NO}_2$ . Teledyne, recognizing this serious deficiency, attempted to rectify this problem by the addition of a cellulose acetate membrane that is impermeable to water vapor and permeable to  $\text{NO}_2$ . Our studies have confirmed the validity of this approach. Figure 8 shows the responses of an old and a new oxidizer transducer when exposed to room air varying in relative humidity from 0 to 100 percent (at 23 C). The curve for the new transducer remains essentially flat with the exception of small excursions immediately upon transfer from low to high to intermediate levels. The old transducer, however, showed marked deviations from the baseline and required extended time periods to return to the baseline.

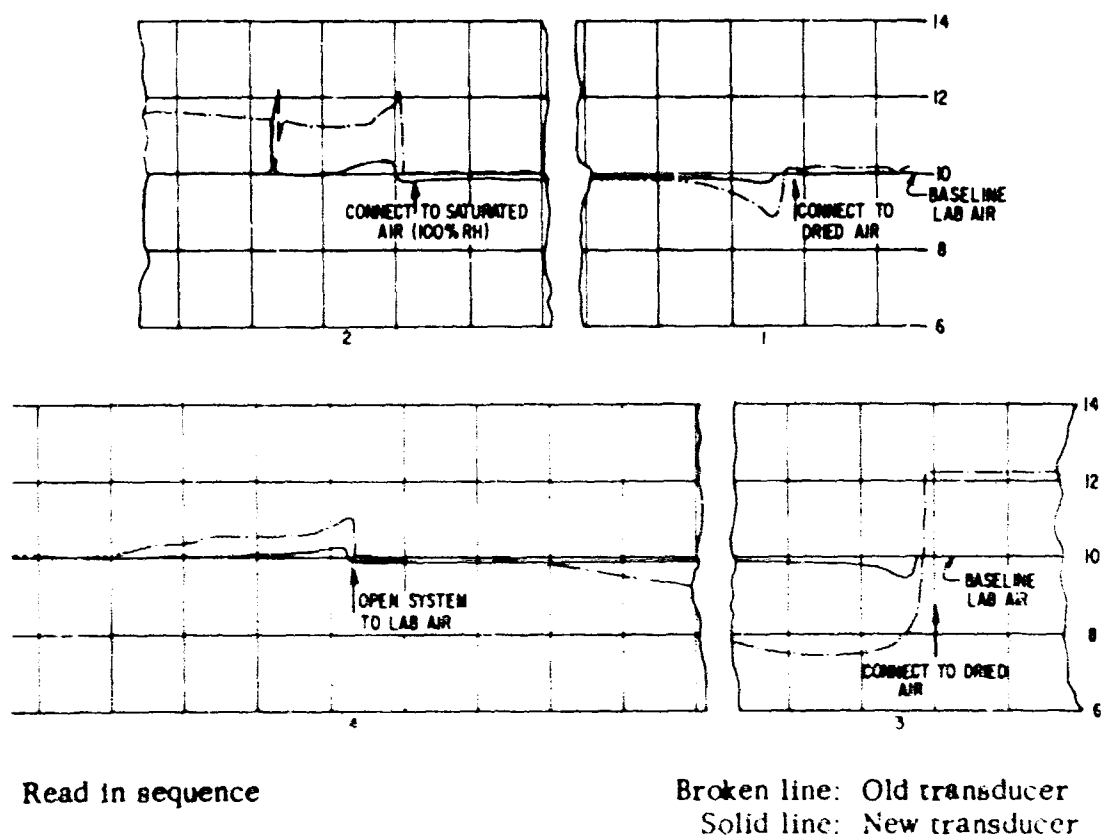


Figure 8. OXIDIZER TRANSDUCER RESPONSE TO WATER VAPOR

## FIELD COMPARISON OF THE OLFACTRON AND COLORIMETRIC DOSIMETER

A complete evaluation of any device destined for utilization in the field must include testing under actual use conditions. A preliminary study of the efficacy of the MSA colorimetric dosimeter was conducted at Picatinny Arsenal, Dover, New Jersey, in the static test stands and related facilities of the Rocket Engine Section. Badges were placed on the lapels of individuals working in various areas and readings were obtained periodically by comparing the color developed on the bindone sensor strip with the color standards. Tables VI-VIII contain data representative of several studies performed during normal engine testing operations over a period of five weeks. The personnel who entered the test cell worked on various parts of the experimental engine and performed tasks such as connecting fuel lines and preparing test equipment. The data in table VI show that the first noticeable exposure occurred after three hours.

TABLE VI

## BINDONE DOSIMETER RESPONSE DURING MHF-3\* TESTING

Observation Time, hr.	Response (CT) in ppm-minutes					
	Subject:	1	2	3	4	5
0.5		0	0	0	0	0
1.5		0	0	0	0	0
3.0		200	150	200	150	200
4.0		200	150	250	150	250
5.0		200	150	300	150	250
6.0		200	150	400	150	250

\*Mixture of 84% MMH and 16%  $N_2H_4$ .

Subjects 1 and 2 prepared mixture; Subjects 3, 4, and 5 worked in test cell and immediate area

TABLE VII

## BINDONE DOSIMETER RESPONSE DURING UDMH ENGINE TEST A

Observation Time, hr.	Response (CT) in ppm-minutes					
	Subject:	1	2	3	4	5
0.5		0	0	0	0	0
1.5		0	0	65	130	65
3.0		0	0	80	165	130
4.0		65	65	80	165	165
5.0		130	80	80	165	165
6.0		130	130	80	165	165

All subjects worked in the test cell and control room

TABLE VIII

## BINDONE DOSIMETER RESPONSE DURING UDMH ENGINE TEST B

Observation	Response (CT) in ppm-minutes				
Time, hr.	Subject:	1	2	3	4
1.0		0	0	0	0
3.0		65	200	80	0
4.0		80	215	130	50
5.0		130	270	130	80
6.0		130	500	215	130

All subjects worked in test cell and control room

Assuming that the entire exposure occurred within 5 minutes, the maximal exposure was 260/5 or 40 ppm which is less than the EEL of 90 ppm for 10 minute exposure for MMH. The value of 400 ppm-min is also of no toxicological significance; hence the dosimeter responses indicated that the test procedure was conducted without any danger to the test personnel. In another test with UDMH, the dosimeter responses were all within acceptable levels, as shown in table VII. Both sets of data further indicate that maximal exposure can be readily observed as evidenced by the lack of further darkening of the sensor strips. Table VIII shows that 500 ppm-min was the highest value observed during these tests. Again, if one assumes that the total exposure indicated during the fifth and sixth hour occurred in the first five minutes, this represented only 230 ppm-min (500-270), which is below the 10 minute EEL of 90 ppm. Thus this cursory study indicates the validity of the use of the colorimetric dosimeter; however, peak concentrations are not discernible and a warning alarm is not possible.

To assess the value of both an automated device and the colorimetric dosimeter, a study was conducted at the Picatinny Arsenal during an engine test and filling of a fuel reservoir. Twelve bindone sensor strips were placed at appropriate positions in the control room, shop, and test cell areas. Test personnel wore a dosimeter badge at their breathing zone (clipped to lapels) during the test. The test engine was fueled with UDMH and red fuming nitric acid (RFNA). A portable Olfactron model 6010 that had been calibrated previously was used to monitor all the test areas on an intermittent basis. The entire test lasted for 2.5 hours during which time the bindone sensor strips were observed at 30 minute intervals. At the termination of the test, the strips were carefully examined. The results were as follows: none of the strips placed throughout the test area showed more than the minimal color change (65 ppm-min). Of the six dosimeter badges worn by the test personnel, one showed a 65 ppm-min exposure after 1.5 hours which did not darken thereafter and one showed a sequence of color change of 100, 180, 180, 180, and 200 ppm-min. At no time did the Olfactron indicate more than 1 or 2 ppm for very short durations.

The refilling test was more informative. In this procedure, UDMH was pumped from a 55 gallon drum to a large reservoir. The pump and the drum were outside while the reservoir was located within the test building. Two individuals donned polyvinyl chloride suits with a full head covering that protected the shoulders and chest. Dosimeter strips were placed under and on top of the head protector as well as at random locations around the pumping site. A third individual wearing protective clothing monitored the work area with the Olfactron 6010.

The filling operation lasted approximately 10 minutes during which time the Olfactron was moved around the site at various distances from the pump and 55 gallon drum. The sensor strips removed from under the protective hoods at the breathing zone showed a marginal color change indicating less than 65 ppm-min. The strips placed on top of the hoods at the breathing zone level indicated an exposure of 300 ppm-min or an overall concentration of 30 ppm. The bindone strips placed randomly in the work area showed color changes indicating CT values of 100, 300, and over 1200 ppm-min. The Olfactron readings varied from 2 ppm to off scale (greater than 250 ppm), depending upon location and time of sampling. An obvious influence on the Olfactron response was wind direction and intensity.

These observations indicate rather clearly that field operations are characterized by changing conditions that warrant a dual approach to the monitoring of the work environment. Both the automated, continuously operating instrument and the integrating colorimetric dosimeter provide industrial hygiene data that enable the assurance of safe working conditions and provide safety personnel with necessary information. Neither device alone can accomplish the total function as our data indicate.

## CONCLUSIONS

For almost 10 years attempts have been made to develop instrumentation capable of performing a monitoring function for liquid missile propellant operations. The pertinent literature and our evaluations reveal this attempt has met with minimal success. None of the devices that have been tested have fulfilled field requirements for MMH and NO<sub>2</sub> monitoring. Our studies with the Olfactron Model 6110 show this instrument to be deficient in several respects: 1) field calibration with UDMH for MMH detection cannot be accomplished as designated by the manufacturer, and 2) the reproducibility of the estimation of repeated exposures is limited to short term events and cannot be accepted for operational situations. The acceptance of any device must be predicated upon verification of the field utility of the instrument under all contemplated operational conditions.

The constraints established by the system to be monitored warrant that judicious application of sound industrial hygiene techniques be controlled and monitored. Our field data indicate that integrative techniques as well as real-time estimates of concentration are required capabilities. In the absence of adequate instrumental methods, emphasis should be placed on those methods that have proven reliability, namely, the colorimetric procedures. Thus the discussion referred to earlier is still appropriate. In conjunction with the sensitivity of olfactory detection as shown by Rumsey and Ceta (1969)--odor thresholds of less than 0.5 ppm for NO<sub>2</sub> and less than 0.3 ppm for UDMH--the colorimetric tubes (Scott-Draeger) can provide useful information until instruments and dosimeters are developed that have the requisite reliability.

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## BERYLLIUM ANALYSIS BY GAS CHROMATOGRAPHY

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### INTRODUCTION

The toxicity of beryllium metal and certain beryllium containing compounds is a familiar problem which has confronted industrial hygienists and toxicologists since the 1940's. Much research has been performed in attempting to identify the hazardous forms of beryllium and the physiologic mechanism(s) by which it exerts its toxic effects (for a review see Stokinger, 1966). Methods for detecting minute amounts of beryllium have been developed and used throughout the years by researchers. Emission spectrography and morin spectrophotofluorescence have been widely used. These techniques are capable of detecting small amounts of beryllium (0.003  $\mu$ gm and 0.01  $\mu$ gm respectively) yet they have marked disadvantages - large samples are required as well as extensive sample preparation. In certain research areas, particularly biological research, large samples are not available. In this laboratory the search for a new, ultrasensitive technique for detecting and quantitating beryllium in biological materials resulted in the development of a gas chromatographic method of analysis. In a recent publication we reported in vitro studies in which the gas chromatographic technique was used to detect and quantitate as little as 0.295 micrograms of beryllium in a 0.05 ml sample. We wish to report here the first successful application of the gas chromatographic method to the analysis of blood and tissues obtained from rats administered intravenous beryllium sulfate.

### MATERIALS AND METHODS

The gas chromatographic method of analysis was published previously (Taylor et al, 1968) and is summarized below:

1. Pipette 50 microliters of blood sample (or a 0.1 ml aliquot of tissue homogenate) into a glass ampule\*

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\*disPO<sup>®</sup> Pipets were used to fabricate the ampules

2. Add 0.5 milliliter of a benzene solution containing 1  $\mu$ l of trifluoroacetylacetone\*
3. Flame seal ampule, allow to cool, shake vigorously, and heat at  $110 \pm 5$  C for 15 minutes.
4. Allow ampule to cool to room temperature and open, add 20 microliters of 28% ammonium hydroxide and agitate for 10-15 seconds.
5. Stopper ampule and centrifuge at 5,000 rpm for 10 minutes.
6. Chromatograph 0.5 to 1 microliter samples of the benzene layer on 6 ft glass column packed with 10% SE52 on Gas-Chrom Z. Column temperature = 90 C, Injector temperature = 140 C, Detector temperature = 180 C. Nitrogen Flow = 100 cc/min, electron capture detection. Varian Aerograph Model 2100 Gas Chromatograph was employed.

White, male laboratory rats (Charles River Laboratories) weighing 350-400 gm were employed in these experiments and were fed food and water ad libitum. The rats were anesthetized with ether and given 2.3 mg ionic beryllium per Kg body weight intravenously (tail vein). One hour after injection the rats were reanesthetized and, using the eye method, about 1 milliliter of blood was collected in either a plastic or silinized glass vial containing 2 drops of heparin solution. The rats were bled in the same manner at 3 hours post injection. At 24 hours post injection the animals were bled as before and while still under ether anesthesia the spleen and a portion of the liver were excised. Homogenates of the harvested organs were prepared by grinding 200-300 mg of tissue with an equal volume of 1M acetate buffer. Blood was analyzed without dilution or preparative measures. Values given in table I are the average of two or three analyses performed on each blood or tissue sample.

TABLE I

MICROGRAMS BERYLLIUM PER ML BLOOD OR PER GRAM TISSUE FOLLOWING  
I. V. ADMINISTRATION OF 2.3 MG BERYLLIUM PER KG

<u>Rat #</u>	<u>1 Hour Blood</u>	<u>3 Hour Blood</u>	<u>24 Hour Blood</u>	<u>Liver</u>	<u>Spleen</u>
1	2.4	2.2	0.05	.80	2.1
2	2.0	1.5	ND	0.451	1.3
3	1.5	1.0	< 0.05	ND	1.5
4	2.5	1.6	<0.05	0.21	0.86
5	5.1	4.2	0.30	0.10	5.2
6	2.4	2.4	0.10	0.05	2.7
7	1.9	1.3	0.06	0.10	2.4
8	2.7	1.6	<0.05	0.10	1.9
9	2.4	1.6	0.07	0.09	2.3
10	2.6	1.8	0.05	0.11	1.8
Controls(5)	ND	ND			ND

\* In this series of experiments  $0.415 \times 10^{-6}$  g of tetrabromoethane was added as an internal standard.

In a second experiment male rats weighing 450-600 g were injected with 5 mg ionic beryllium per kilogram. Animals were bled 1/2 hour post injection and again at 24 hours post injection at which time liver samples and spleens were taken for analysis. The analyses of these tissues gave the following results:

TABLE II  
MICROGRAMS BERYLLIUM PER ML BLOOD OR PER GRAM TISSUE FOLLOWING  
I. V. ADMINISTRATION OF 5 MG BERYLLIUM PER KG

<u>Rat #</u>	<u>1/2 Hour Blood</u>	<u>24 Hour Blood</u>	<u>Liver</u>	<u>Spleen</u>
1	2.9	0.68	5.2	2.3
2	2.8	0.80	4.1	*
3 (Control)	ND	ND	ND	ND
4	1.3	*	0.94	*
5	5.8	0.35	3.8	3.4
6	1.6	*	1.1	1.0
7 (Control)	ND	ND	ND	ND

\*Samples deteriorated prior to analysis.

#### DISCUSSION

The results presented in tables I and II indicate the beryllium in tissues obtained from rats administered intravenous beryllium sulfate can be determined using the gas chromatographic technique. It is surprising that the rats are apparently capable of removing such a large percentage of the injected beryllium so rapidly. It is also surprising that the results obtained in the first experiment (2.3 mg/Kg) and the second experiment (5 mg/Kg) are not significantly different. In other words, a more than twofold increase in dose failed to markedly boost the beryllium levels found in the blood and outlying organs. This is further illustrated by the graphic presentation of beryllium levels in blood vs time shown in figure 1. The curve shows the decline of beryllium levels in blood following administration of 2.3 mg/Kg ionic beryllium. Points on the curve were obtained by averaging all values obtained at 1, 3, and 24 hours. The points indicated with an X at 1/2 hour and 24 hours are the average of all values at the respective time intervals following injection of 5 mg ionic beryllium per Kg. Vacher and Stoner (1968) working with ionic beryllium solutions made radioactive by the addition of  $^{70}\text{Be}$  obtained the results shown in figure 2. These workers postulate that the mechanism(s) of the rat for removing Be vary according to the levels introduced. According to these workers at least two mechanisms are responsible for the removal of beryllium and the extent to which each of these mechanisms is involved in the removal of beryllium is dose dependent. At levels below 0.03  $\mu\text{g Be/Kg}$  and above 0.5 mg Be/Kg Vacher and Stoner observed that the initial rate of removal of

beryllium from the blood is extremely rapid. Our results seem to be in agreement with the findings of Vaher and Stoner in that we see an extremely precipitous falloff of beryllium levels at both 2.3 and 5 mg per Kg. Furthermore, it seems that the similar beryllium levels found in blood following 2.3 mg and 5 mg per Kg can be explained by postulating that the initial mechanism for removing beryllium at doses higher than 0.5 mg/Kg is extremely efficient and rapidly removes beryllium from the blood until a certain equilibrium is attained after which the second mechanism continues to remove the beryllium at a much slower rate. Since our primary concern is the development of an analytical technique and not a distribution study, we did not attempt to verify the above hypothesis. Work is currently underway in our laboratory to check the accuracy and precision of the gas chromatographic technique by using a radiotracer technique similar to that of Vacher and Stoner. Preliminary findings indicate excellent agreement between the two techniques.

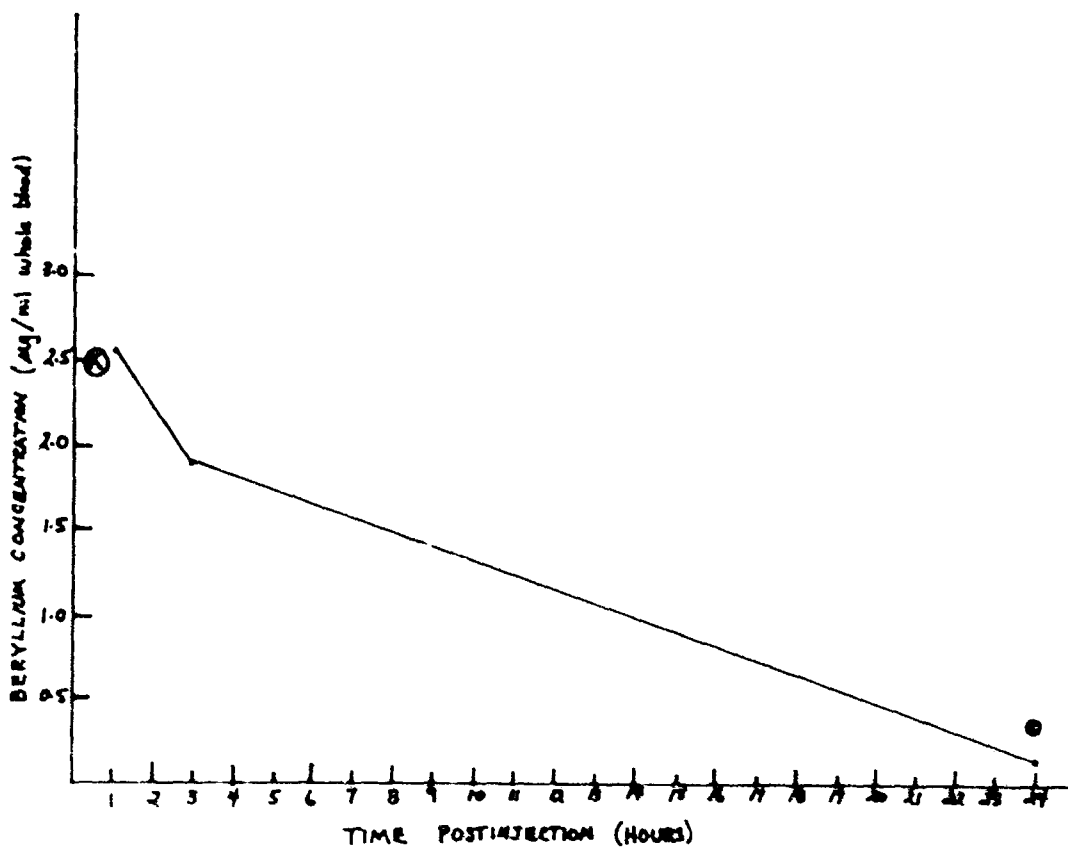
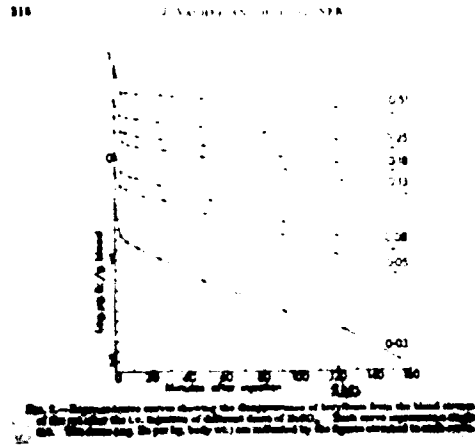


Figure 1. BERYLLIUM CONCENTRATION IN RAT BLOOD VS TIME POST INJECTION



### CONCLUSIONS

1. Beryllium administered as aqueous beryllium sulfate to male rats can be detected and quantitated in the blood, liver, and spleen up to 24 hours following injection of either 2.3 mg per Kg or 5 mg per Kg.
2. The sensitivity of the gas chromatographic method for quantitation of beryllium is 0.05 micrograms per milliliter of whole blood.

### REFERENCES

1. Stokinger, H. E., ed; Beryllium Its Industrial Hygiene Aspects; Academic Press, New York, 1966.
2. Taylor, M. L., L. L. Arnold, and R. E. Sievers; "Rapid Microanalysis of Beryllium in Biological Fluids"; Anal. Lett., 1:735-747, 1968.
3. Vacher, J. and H. B. Stoner; "The Removal of Injected Beryllium from the Blood of the Rat, the Role of the Reticuloendothelial System"; Brit. J. Exp. Path., 49: 315-323, 1968.

## DISCUSSION

MR. MOBERG: Have you tried hexafluoral acetone, or do you have any of the material?

CAPTAIN TAYLOR: I feel that it would probably be too volatile and would elute too fast so that you wind up having it elute where the crud is, if you will excuse the expression. Of course you could change your column technology and perhaps make good use of it, but we had a good thing going so we wanted to see this through.

MR. MOBERG: The reason I made this comment is, or even if you substituted one of the fluorines with a bromine, you might have less volatility but you might get much more flame response, and consequently aid you to get further sensitivity.

CAPTAIN TAYLOR: Ross and Sievers have done work along this line, and you are quite right; however, the benefit is not twofold, as you might expect. They also have done work with other chelating agents containing as high as seven fluorine atoms per molecule, and they realized some advantage, but there are some other disadvantages such as the partition coefficient between chelated beryllium and the organic layer versus water, and the stability, the tendency to form hydrates and so forth, but again these things could be lived with if there was a need to go to that.

MR. MOBERG: Very good. I might ask Captain Taylor if he would care to comment on the conversion of beryllium oxide to a form that might be useful in using this methodology because certainly this is of prime interest to the rocket people. The oxide, of course, it is common also in metallurgical industry. Would you care to comment on that?

CAPTAIN TAYLOR: Yes, of course this has been on our minds for some time. We are trying to make a sane and progressive approach to this. I would call your attention to the fact that we synthesized our chelated beryllium from elemental beryllium powder laying in the bottom of the flask. We then added the chelating agent to it and you can see the hydrogen evolve as it reacts; so this should give you a feeling for the affinity that this chelating agent has for beryllium. We have done, then, cursory studies where we took low-fired beryllium oxide, a sample obtained from Dr. Spencer at Dow Chemical Company, and sealed it in an ampule and heated it at 37 degrees. (I was curious to see if we could get a reaction under a sort of physiological condition.) We then analyzed the organic layer in the manner described and got a beryllium peak. Now there are a number of holes that could be picked in this one of which is: How do I know this is beryllium oxide that is reacting and not entrapped

ionic beryllium in the sample? Well, I don't, except that this material had lain for some time in an aqueous medium. I filtered off the insoluble beryllium oxide and threw it in with the chelating agent, so perhaps I have partially answered that one question. Dr. Sievers, who has been kind of a father-figure for this work, assures me that the binding constants indicate that there will be no problem. High-fired beryllium nitrate or nitride may be another ball game. This is a high-fired ceramic material and that may be a toughy, but yet based on the voracious appetite of the chelating agent for beryllium that we have seen so far, we may see success. We haven't tried that at all.

DR. CAMPBELL: Does the beryllium atom contribute anything to the electron affinity of the chelated molecule?

CAPTAIN TAYLOR: Yes. Ross and Sievers feel that the metal in question does have a profound effect on the results. This gets into some intricate features of column technology. Some French workers, in what would correspond to their Atomic Energy Commission, have done quite a bit of work on trying to find aluminum in uranium samples, and due to this, they have done some rather meticulous work finding out where the aluminum goes in the column, and they find that it behaves differently, depending upon the metal. I think it is also true, to answer your question directly, that the metal atom does play a part.

FROM THE FLOOR: I would be interested in hearing the philosophical reasons as to why you get the same plasma levels at two different dose levels of beryllium. If taken at face value this is quite interesting because it suggests the volume of distribution of this material is dose dependent. I think it has a quite interesting implication.

CAPTAIN TAYLOR: Well I agree with you. I'm not sure whether you are for me or against me, but I'm glad you asked the question because it should be asked and I spent a great deal of yesterday in frenzy trying to get my paper together on this. Bocker and Stoner have summarized very nicely the work done in recent months and years on the particular facets of what happens to ionic beryllium when you inject it in an animal. And if you take what they say at face value, what they say is that the beryllium is converted into two types of material when injected, a beryllium phosphate with a so-called insoluble form, and beryllium complexes with citric acid and other commonly occurring acids which they call a soluble form. They didn't go ahead and say that they have shown at least to their satisfaction that certain of these particles are protein coated. Alpha globulin has been implicated where the beryllium phosphate is concerned. They have done elaborate studies showing that the reticuloendothelial system participates in scrubbing out beryllium phosphate and protein-bound beryllium phosphate. They further say that they feel, depending upon the dose, they get various types of protein aggregates formed. In other words, a high dose will give a range of particulates, if you will, ranging from big molecules, large protein-bound molecules, down to small ones, and they then feel that the large ones are removed faster and the smaller ones are removed slower. Now in an effort to equate some of these "known" facts, I feel at the levels where we are, we have "shot gunned" the animals, we have given him a whale of a dose, 5 milligrams per kilogram is ten times the LD-50 for ionic beryllium; so we have overloaded his system to the extent that he is a very sick



animal, and all removal systems I feel at this point are highly saturated. So that until we get down in the sub-milligram per kilogram range, we won't really see a marked difference in the curves. All the work that I was able to locate was not similar enough to our work to really throw a curve up here and say we got this and it agrees with them. Most people are using beryllium-7 and it is interesting, that carrier-free beryllium-7 if given, in very very small quantities, it is removed at a different rate from the labeled beryllium sulfate; so that Bocker and Stoner in their studies showed that the carrier-free material is removed at a much faster rate initially than is the labeled material. They also found it on the high end of the spectrum at the LD-50 level. They also saw again this steep rate of initial removal. The intermediate dose levels were more or less the same; so, they hypothesized that there were two mechanisms occurring which contributed to the removal. I don't know whether I have answered your question or not, but it is interesting to kick this thing around, and we hope to bring the technique to the point where we have no question of its validity and its ability to perform some of these things.

CAPTAIN ARNOLD (Aerospace Medical Research Laboratory): In the two series of experiments that we have done with the beryllium in vivo, Captain Taylor showed you that there was a great deal of similarity between the levels we found in the blood both early in the intoxication and at 24 hours afterward after the admission of the dose. These blood levels seemed to be pretty similar regardless whether we ran five milligrams per kilo, or whether we ran two milligrams per kilo; however, when we started looking at the livers and spleens there seemed to be a significant difference in the levels between the two doses that we find in there; namely, that in the light dose, the livers didn't seem to have a very high level of beryllium in them. The spleens were quite high. At the higher dose, the five milligram dose, it seemed to be a reverse of the phenomenon. In other words, the liver seemed considerably higher than the spleen.

CAPTAIN TAYLOR: And in this connection I would say it appears that the reticulo-endothelial system has been wiped out at the five milligram per kilogram dose.

MAJOR MAC KENZIE: I'm going to add a comment. I looked at these tissues. At the low dosage at 24 hours, the only lesion is in the Kupffer cells in the liver. The nucleus is karyorrhectic. From the light microscope view, the hepatocytes appeared normal. In the higher dose all hepatocytes show rather serious signs of early necrosis. I have read these "blind", and it is rather typical for you to pick it out so there is a difference in the pathology as well as in the level. You are dealing with two different situations. This is a long chronic disease and we are reading these at 24 hours. There are two different diseases with beryllium, one is the chronic berylliosis and the other is acute toxicity.

MR. MOBERG: All right, we will pass on. How about the work of Ray Saunders on dichloroacetylene? I think that the chemist has been using many abbreviations, MCA, DCA, FID, EC, and Dr. Thomas said: "Wait a second, tell us what you are talking about", I was afraid to return the comment because a chemist just listening to a medical meeting really gets the shock of his life in the terms. MCA is mono-

chloroacetylene and might have been mentioned this morning. These products occur as you dehydrohalogenate, remove HCL, from heated solids like lithium hydroxide, and remove the proton and the halogen, and you can come down to a totally unsaturated acetylene form. Trichloroethylene is not the only beast that will make these components. Dichloroethane and the other halogenated solvents and some of the freons can also produce very noxious materials, so that we would warn you not only to leave out trichloroethylene but maybe dichloroethane and maybe dichloroethylene as a cleaning solvent. These are all vulnerable solvents.

DR. AZAR: I would like to comment on Mr. Saunders' paper and ask that whenever they do the post on this monkey they do tissue level of dichloroacetylene and see how these compare with the data that Captain Siegel has in tissue levels.

MR. MOBERG: That's a good comment. I'm not sure that I can field it all for Ray, but I would add that the animal that reportedly was exposed to high levels of dichloroacetylene has already been pretty much covered. The comment I made about official reporting of the data is in reference to the flight of Bonnie, in that case our analytical data from the atmosphere, and from the charcoal desorption studies did not indicate dichloroacetylene present. So I'm not sure if it is too late for that early animal study to go back and ask them to do that, or suggest it to them.

OPEN FORUM

MR. MOBERG: I would like to proceed now for questions on the other papers on the environment in the home. Questions or comments may be directed to Dr. Longley.

DR. AZAR: I was wondering if Dr. Longley would care to elaborate a little more as to where he was sampling from, and the condition of exposure when he detected a hundred parts per million of halogenated hydrocarbon?

DR. LONGLEY: We were sampling from the return air duct of the forced air vent system. What conditions do you have in mind? What conditions were you referring to?

DR. AZAR: You mentioned the individual in the home used a hairspray. I was wondering if you were sampling from the bedroom or the bathroom, or, this seemed like a fairly high concentration to achieve just on one episode of using a hair spray, if you were sampling from the whole house.

DR. LONGLEY: On this particular one, we were estimating about a little bit over a half an ounce of the aerosol used, and the release was in one of the bedrooms, and we were sampling continuously from the return air duct which takes air from all over the house. Now this is a hundred parts per million and was a peak which existed for some number of minutes, then slowly degraded to a point where we were still measuring some of it four hours later. I'd make an estimate of about ten parts per million.

DR. HODGE: I understood that this was a tracer experiment in which this material was deliberately released in order to follow patterns and concentrations, not an in-use sort of concentration that you get in the normal home. Was I mistaken?

DR. LONGLEY: No, we were conducting a trace experiment to find out infiltration rates. The accidental release was done inadvertently by one member of the household. She, in effect, loused up our measurement!

MR. MOBERG: How about comments or questions on the blood catecholamine measurements?

MR. BUCK (Arthur D. Little, Inc.): This is in the nature of a comment rather than a question. I think that the recoveries which were reported, one hundred percent at two milligrams per ml were quite unfair. Not infrequently it happens that percent of recovery goes down dramatically when concentrations go down. The 70 percent recoveries reported for the fluorometric methods are really quite good, all things considered. This is micrograms per ml, and I think if you succeed in doing this

you will be in fact doing very well, even with the sensitive techniques that you have.

MR. MOBERG: I think one of the reasons they did this was because of interferences. They had quite a bit of interference from other components with the fluorometric method, at least this was a comment that was passed on to me. I would be just a little afraid that you might chelate other alcohols that would also go into the filtrate or hydroxy groups or not chelate them but form a derivative and possibly this might interfere with the chromatograph if you didn't have any quantitative backup from the mass spec. Would there be any comments on that?

CAPTAIN ARNOLD: Undoubtedly these alcohols do form derivatives. These are well known. They form nice stable esters and these esters are chromatographed. However, you must realize that with alcohol, say ethanol or propanol, there is a great difference in molecular weight and, consequently, a great difference in boiling point between these compounds and the derivative that I am actually working with. You get up to the TFA derivative of epinephrine, for instance, and you've got a compound that is running up around 500 molecular weight and it takes quite a little while to get off of that column. Opposed to this, with lower molecular weight alcohols, they pretty much come off at the same time the solvent does.

MR. MOBERG: You mean even the alcohols C-6 to C-12? or the hydroxic groups? even ethylene glycol?

CAPTAIN ARNOLD: I would say with ethylene glycol, you start getting up into the really higher molecular weights. These could be a problem; however, I don't think that you are going to find them normally in the method of extraction that we go through. In other words, throw-away acid conditions, due to the TFA trifluoroacetic acid extraction, this will eliminate quite a number of interferences.

MR. MOBERG: I really pose these problems constructively, not as criticism.

CAPTAIN ARNOLD: I might comment on the past comment that I didn't mean to imply that I was expecting this kind of yield once I get down to the nanogram or picogram levels. I realize that as you go down in concentration you do lose some of your recovery.

MR. MOBERG: Thank you. Any other comments or questions on this work?

Finally, the work that was reported on the field instrument measurement for  $\text{NO}_2$  and the hydrazines. Are there any questions or comments on this?

MR. VERNOT: Just one comment. You recommended, Dr. London, pretreating the detector and then letting it rest a while before you used it for analysis. Is there a danger that when you are using it for analysis you are retreating it again and then adding some instability to the thing?

DR. LONDON: If I said pretreatment, I didn't mean pretreatment. I meant calibrating, and then letting it set. Our experience has been after 48 hours, the transducer reads what we believe the concentration to be. Now, in order to insure that in fact that is what is happening, we will have to get multiple numbers of transducers, calibrate them all at some level, and then let them rest and expose each individual transducer to high levels, low levels, and presumably in the face of what we think we know, we should hit the established concentration. But I don't think after this 48 hour rest period that anything would happen after that.

FROM THE FLOOR: We saw a phenomenon that as you loaded this transducer, say ten to twenty-five times within one day, your response was decreased. We let it rest 24 hours until the next morning and took it right down to one concentration. It read it, but as you continually expose it more and more its response reduced--Just like a human.

The whole theory for the use of the instrument is that you are going to be walking from an outside environment into a silo or area supposedly with a leak. You want to read it correctly the first time, and all we are saying is, don't expect it to read accurately after that first reading if you keep on exposing it.

MR. VERNOT: O.K. I'm sure it will work that way, but you won't be able to get a continuous measurement for any period of time.

DR. LONDON: This is why I attempted to emphasize the necessity of using an integrative device like a colorimetric dosimeter to assure that during an EEL exposure of an individual, participating in whatever has to be done, registered the actual dose he has been exposed to.